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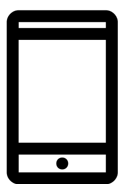

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Dear TERMIS EU 2022 Participants,

On behalf of the scientific committee, it is our great pleasure to welcome you to the **Tissue Engineering and Regenerative Medicine International Society (TERMIS) European Chapter Conference 2022** in Krakow, Poland, from 28th June to 1st July 2022.

We are delighted that the conference has received a lot of interest from participants and are happy that we could organize TERMIS EU 2022 as an inspiring four-day face-to-face scientific meeting. This is the first TERMIS meeting to be held face to face post the pandemic. So we all have a lot of catching up to do at a professional and personal level.

We have done everything we could to provide researchers and scientists with an opportunity to get together, network, learn and adopt innovative thinking in the exponentially growing field of tissue engineering and regenerative medicine. The state-of-the-art 5 plenary lectures are complemented by 95 invited keynotes and 68 scientific theme-oriented sessions related to the conference topic **"Perspectives and Challenges in Regenerative Medicine."**

We are also providing several activities for Students and Young Investigators, including social networking night, best poster competition, meeting with mentors and debates.

This is the first time the TERMIS-EU conference has been held in Poland, in the second-largest and one of the oldest cities in Poland, Krakow. It is a historical and cultural center, a university city, and a hub for developing new technologies.

In these challenging times of conflict around our borders, we stand in solidarity with all the scientists who have been affected. We have made every effort to support those who were affected by this conference.

We hope you will enjoy the Conference and Krakow city!

Prof. Wojciech Święszkowski

TERMIS-EU 2022 Conference Chair
Warsaw University of Technology

Prof. Zygmunt Pojda

TERMIS-EU 2022 Conference Co-Chair
Maria Sklodowska-Curie National Research Institute of Oncology

Dear TERMIS-EU 2022 Participants,

Our TERMIS-EU 2022 meeting is remarkably important and worthwhile in so many ways. We continue to live in a troubled world, yet the fact that you are now gathering in Kraków sends a double message of hope and positive opportunity to our community and beyond. I am particularly delighted that this is the first face-to-face meeting that TERMIS has held since before the pandemic. TERMIS-EU is privileged to lead our efforts to return to a more traditional style of meeting. I acknowledge Professor Abhay Pandit as TERMIS-EU Chapter Chair and thank him and all the team for their ongoing work and making this a reality.

TERMIS is the most prominent organisation in the field of tissue engineering and regenerative medicine globally. Among its activities, TERMIS promotes education, research, innovation, clinical translation and social responsibility within the field of tissue engineering and regenerative medicine through regular meetings, training courses, scientific and lay publications, outreach activities and more. TERMIS provides an important international forum for the informed discussion and debate of achievements and challenges of tissue engineering and regenerative medicine therapies.

TERMIS relies on countless contributions by outstanding people who freely give of their time and skills to help our not-for-profit Society. Through its three Chapters, TERMIS functions to broadcast superb tissue engineering and regenerative medicine for the broader benefit of the world. I thank our remarkable and indispensable Sarah Wilburn, fine Chapter Chairs and TERMIS officers, dedicated committees, students and young investigators, our countless volunteers, our members, supporters, industry partners, donors, and more. All of you help to strengthen TERMIS, for now and in the many years ahead.

As participants, you are so fortunate to enjoy Kraków. It is a beautiful setting for a special meeting. A double thank you to Professor Wojciech Swieszkowski and Professor Zygmunt Pojda and your teams for your hard work towards this fine conference.

I wish you all the very best. Renew your friendships, discover new colleague, share experiences, discuss exciting discoveries, and contribute to a better world. Enjoy our TERMIS-EU meeting and make sure you celebrate in this great city!

Professor Tony Weiss

TERMIS Global President

Dear Colleagues,

A hearty welcome to the TERMIS-EU 2022 meeting!
On behalf of the Tissue Engineering and Regenerative Medicine International Society-EU Chapter Meeting, I would like to welcome you to Krakow for what promises to be an exciting and informative conference. The theme of the meeting is 'Perspectives and Challenges in Regenerative Medicine'. As you are all aware, the last couple of years have been challenging for all of us.

The local organising committee has worked diligently to make this meeting a reality despite all the odds. I would encourage all of you to take time in your busy schedule to thank the organisers personally for bringing us together. We are at the crossroads of new understandings at cellular and molecular levels coupled with interesting concepts in biofabrication technologies with advances in the understanding of immunology, always remembering our ever-present need to drive existing technologies towards clinical translation. The conference programme is designed to reflect these developments and challenge some of the existing paradigms, helping to inform the field's future direction.

As you can see, it will be a hectic meeting! We hope that this meeting will get you excited about new directions in the field with excellent plenary speakers, a FTERM debate, top-notch symposia, renowned keynote speakers, podium presentations and poster presentations, giving you an opportunity to interact with your colleagues from around the world. A meeting like this could not be organised without the work of many individuals and groups. A thank you to all speakers, exhibitors, committee members, sponsors, reviewers, the TERMIS-EU council and the outstanding work of the local organising committee led by Prof. Wojciech Swieszkowski and Prof. Zygmunt Pojda. Special gratitude is due to the committee for providing complimentary registrations and support to our Ukrainian colleagues. Be assured that we at TERMIS have reached out to them and have made every effort to assist them in these difficult times. We will be embarking on a more expanded mission of supporting scholars-at-risk in future meetings.

The TERMIS-EU council has been quite busy in the background over the last two years. We would like to expand our membership and engage industry and clinicians more effectively. Towards that goal, we will soon launch our five-year strategic plan by the end of the year. Thank you to those who have partaken in our surveys—we have heard your voice.

This is a special meeting for us in Krakow. It is the very first meeting of this scale in the field of Tissue Engineering and Regenerative Medicine to be held in Poland. We will remember this for a long time and hope this meeting will be valuable for you. Enjoy Krakow, an exciting and vibrant city with plenty of things to do, night-time dining to suit every taste and places to see. Take home memories that will enrich your professional life and your personal life.

Abhay Pandit

Chair TERMIS-EU

Dear TERMIS EU 2022 Participants,

Ukraine has been invited as the Guest Nation of TERMIS EU 2022. We are truly honoured for this recognition.

Our country has been working in the field of tissue engineering (TE) and regenerative medicine (RM) since the Ukrainian independence. Historically, the Institute for Problems of Cryobiology & Cryomedicine of National Academy of Sciences of Ukraine (NASU) in Kharkiv is known in Ukraine for their pioneering experimental work in cell culturing and cryopreservation, disease modelling. Before the annexation of Donbas region, the State Gusak Institute for Urgent & Recovery Surgery in Donetsk was involved in large-scale clinical use and effects' studying of the living dermal and bone equivalents for treatment of casualties with burns and mine trauma, as well as for patients with trophic ulcers of the lower leg (2003-2014). The modern flagship of RM & TE in Ukraine is the State Institute for Genetic & Regenerative Medicine of National Academy of Medical Sciences of Ukraine (NAMSU), established in Kyiv in 2007. In addition, the research projects and teams of our institute are closely bound with the Institute of Molecular Biology and Genetics of NASU. There are joint projects between our institutes in the area of genetic and regenerative medicine.

The main goals of researches of Ukrainian teams are development of biomedicinal products based on cultured somatic cells and scaffolds, as well as the generation of genetically modified cells to study and assess their therapeutic efficacy for established experimental models of human diseases. Also in Ukraine, in last years, there were developed and clinically applied the tissue-engineered living dermal and bone equivalents for combustiology, traumatology and orthopedics, dentistry and maxillofacial surgery applications.

Funding of research in TE & RM is basically provided through the NAMSU and NASU, as well as the recently established in 2018 National Research Foundation of Ukraine, but there is a small percentage of projects funded by private biomedicinal companies.

There is no special TE education in Ukraine as such. There are only short-termed educational courses of medicinal biotechnologies and regenerative medicine as part of basic university studies, for example, at Bogomolets National Medical University, Biology & Medicine Institute of Taras Shevchenko National University of Kyiv, National University of Kyiv-Mohyla Academy, and Shupyk National Medical Academy of Post-Graduate Education.

Currently, twenty eight Banks of Human Umbilical Cord Blood, other Tissues and Cells are operated in Ukraine. They are all mostly private banks. Of these, a few are engaged in cell culturing, tissue-engineered and cell-based products' manufacturing and releasing for clinical use under the state business license.

The only known case of mass clinical application of the TE bone equivalent is the experience of our institute together with a private medicinal and biotechnology company and a charitable foundation. It was done for the restoration of combat-related critical-sized bone defects in injured soldiers from battlefields of eastern Ukraine. Thus, in the period from 2015 to 2019, bone equivalent grafts were applied to 47 casualties with 49 bone defects. This Ukrainian TE experience in traumatology and orthopedics of the combat injury was highlighted at the TERMIS European Chapter Meeting 2019 (Tissue Engineering Therapies: From Concept to Clinical Translation & Commercialisation) among other global leaders working in the field of bone TE.

Briefly, our pilot clinical study utilized a cocktail of expanded autologous BM-MSCs, periosteal progenitor cells and endothelial progenitor cells on a fibrin hydrogel-DBM composite, to restore critical-sized bone defects of combat casualties with complicated gunshot bone wound. The X-ray examination determined that within 4–6 months post-operatory, 90.4% of the treated defects regained native integrity [1, 2].

Previously, our team conducted a few pilot studies on the clinical use of a living dermal equivalent based on collagen and fibrin hydrogels and cultured ADSCs to support a split-thickness skin auto grafts in the experimental treatment of burned patients (2015-2017, Kyiv City Clinical Hospital No. 2), as well as in the treatment of purulent/necrotic soft tissue defects (2016-2018, Kyiv City Clinical Hospital No. 4). Encouraging clinical results were also obtained by our team in dentistry when using a dermal equivalent composed of fibrin hydrogel, cultured ADSCs and bone chips in gingivoplasty (2017-2018, Department of Dentistry of Bogomolets National Medical University).

Actually, due to the martial law in our country, ongoing TE & RM researches in Ukraine is temporarily suspended or proceed very slowly.
But we are looking to the future. Meanwhile, it would be useful to adopt the practice of the TE biomedicinal products' application by Ukrainian hospitals involved in combat casualty care, e.g., TE living equivalents to restore soft tissues and bone defects in injured defenders of Ukraine.

Dimitri Zubov, Ph.D.

Lead Researcher, Laboratory of Applied Biotechnologies, Cell & Tissue Technologies Unit, State Institute for Genetic & Regenerative Medicine of National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
Head of Biotechnology Laboratory, 'Vitality' Medical & Research Center – ADONIS Group of Medical Companies, Kyiv, Ukraine

1. Sallent, I., Capella-Monsonís, H., Procter, P., Bozo, I.Y., Deev, R.V., Zubov, D., Vasyliev, R., Perale, G., Pertici, G., Baker, J. and Gingras, P. "The few who made it: commercially and clinically successful innovative bone grafts." Frontiers in bioengineering and biotechnology 8 (2020): 952.
2. Vasyliev, R.G., Oksymets, V.M., Rodnichenko, A.E., Zlatska, A.V., Gubar, O.S., Gordiienko, I.M. and Zubov, D.O. "Tissue-engineered bone for treatment of combat related limb injuries." Experimental Oncology 39 (3) (2017): 191-196.

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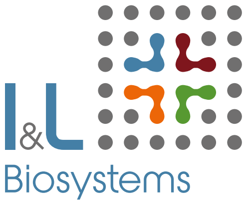

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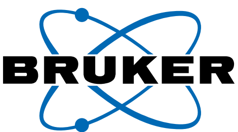

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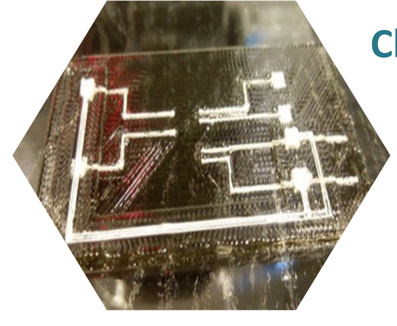

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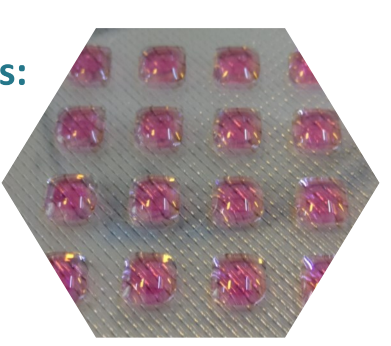

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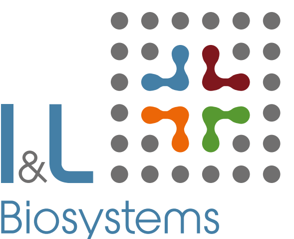

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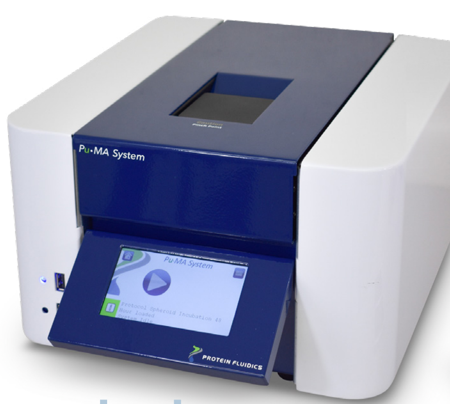

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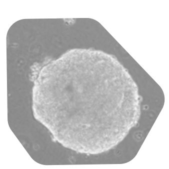

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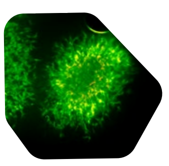

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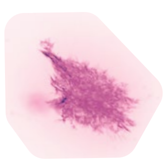

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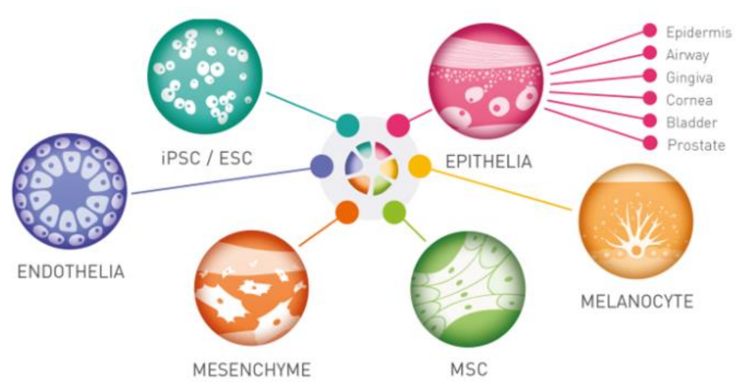

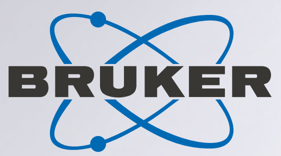

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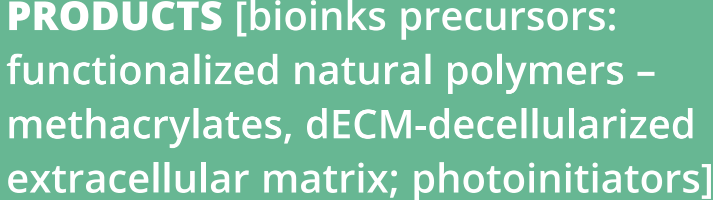

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Stephen Badylak	2012	Americas
Ranieri Cancedda	2012	Europe
Yilin Cao	2012	Asia-Pacific
Manuela Gomes	2021	Europe
Linda Griffith	2012	Americas
Robert Guldberg	2012	Americas
Kiki Hellman	2012	Americas
Jöns Hilborn	2012	Europe
Jeffrey Hubbell	2012	Americas
Dietmar Hutmacher	2012	Americas
Gilson Khang	2012	Asia-Pacific
C. James Kirkpatrick	2012	Europe
Robert Langer	2012	Americas
Jin Ho Lee	2015	Asia-Pacific
Hai-Bang Lee	2012	Asia-Pacific
Kacey Marra	2021	Americas
Ivan Martin	2012	Europe
Alison McGuigan	2021	Americas
Antonios Mikos	2012	Americas
David Mooney	2012	Americas
Gail Naughton	2012	Americas
Teruo Okano	2012	Asia-Pacific
Abhay Pandit	2015	Europe
Nancy Parenteau	2012	Americas
Milica Radisic	2018	Americas
Buddy Ratner	2012	Americas
Heinz Redl	2015	Europe
Rui Reis	2015	Europe
R. Geoff Richards	2021	Europe
Alan Russell	2012	Americas
Katja Schenke-Layland	2021	Europe
Molly Shoichet	2012	Americas
David Smith	2018	Americas
Yasuhiko Tabata	2012	AsiaPacific
Rocky Tuan	2021	AsiaPacific
Charles Vacanti	2012	Americas
Joseph Vacanti	2012	Americas
Gerjo van Osch	2018	Europe
Gordana Vunjak-Novakovic	2012	Americas
William Wagner	2015	Americas
Anthony Weiss	2018	AsiaPacific

TERMIS-EU Council Members

CONTINENTAL CHAIR

- Abhay Pandit

CONTINENTAL CHAIR ELECT

- Manuela Gomes

IMMEDIATE PAST CHAIR

- Gerjo van Osch

MEMBER-AT-LARGE

- Martin Stoddart

TREASURER

- David Eglin

TREASURER-ELECT

- Elizabeth Rosado Balmayor

SECRETARY

- Catherine Le Visage

COUNCIL MEMBERS

- Katja Schenke-Layland
- Lorenzo Moroni
- Ciara Murphy
- Michael Raghunath
- Martijn van Griensven
- Sandra Van Vlierberghe
- Susanne Wolbank
- Lisa White
- Ioannis Papantoniou
- SYIS: Lizette Utomo

2022

Robert Brown Early Career Principal Investigator Award

Riccardo Levato
PhD

TERMIS EU Mid Term Career Award

Marianna Tryfonidou
DVM, PhD

Career Achievement Award

Alicia El Haj
PhD, FREng, FRSB, FEAMBES

2021

Robert Brown Early Career Principal Investigator Award

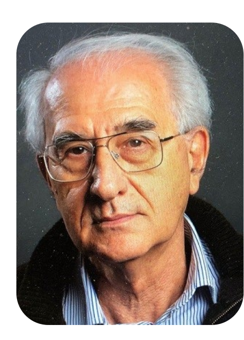
Marietta Hermann
PhD

TERMIS EU Mid Term Career Award

Jos Malda
PhD

Career Achievement Award

Ranieri Cancedda
MD



2020

Robert Brown Early Career Principal Investigator Award

Jeroen Leijten
PhD

TERMIS EU Mid Term Career Award

Martin J. Stoddart
PhD, FRSB

Career Achievement Award

Graziella Pellegrini
Professor of Regenerative Medicine

Angelo Accardo, Netherlands
Mohammad Alkhraisat, Spain
Luigi Ambrosio, Italy
James Armstrong, United Kingdom
Nureddin Ashammakhi, United States
Matthew Baker, Netherlands
Andrea Banfi, Switzerland
Barbara Barboni, Italy
Cristina Barrias, Portugal
Aldo Boccaccini, Germany
Nicolas Broguiere, Switzerland
Guoping Chen, Japan
John Crean, United Kingdom
Andrew Daly, Ireland
Antonio D'Amore, Italy
Patricia Dankers, United Kingdom
Buddhadeb Dawn, United States
Michael Delahaye, United States
Timothy Douglas, United Kingdom
Daniela Duarte Campos, Germany
Józef Dulak, Poland
Mirosława El Fray, Poland
Bernd Giebel, Germany
Cecilie Gjerde, Norway
Manuela E. Gomes, Portugal
Jürgen Groll, Germany
Thomas Groth, Germany
Debbie Guest, United Kingdom
Greg Hudalla, United States
Dietmar W. Hutmacher, Australia
Gunil Im, South Korea
Keita Ito, Netherlands
Diana Jurk, United States
Ryuji Kato, Japan
Daniel Kelly, Ireland
Ali Khademhosseini, United States
Niels B. Larsen, Denmark
Christine Le Maitre, United Kingdom
Catherine Le Visage, France
Riccardo Levato, Netherlands
Shulamit Levenberg, Israel
Malgorzata Lewandowska-Szumiel, Poland
Andrea Lolli, Netherlands
Adam Maciejewski, Poland
Srinivas Madduri, Switzerland
Jos Malda, Netherlands
João Mano, Portugal
Susanna Miettinen, Finland
Farzaneh Moghtader, Iran
Matteo Moretti, Switzerland
Lorenzo Moroni, Netherlands
Francesco Moscato, Austria
Nuno Neves, Portugal
Letizia Nicoletti, Italy
Tomasz Nowakowski,
Fergal O'Brien, Ireland
Mikolaj Ogrodnik, Austria
Aleksandr Ovsianikov, Austria
Sean Palecek, United States
In-Hyun Park, USA
Francesco Pasqualini, Italy
Jai Prakash, Netherlands
Adrian Ranga, Belgium
Gwendolen Reilly, United Kingdom
Rui Reis, Portugal
Daniel Reumann, Austria
José Carlos Rodríguez-Cabello, Spain
Elizabeth Rosado Balmayor, Germany
Margarida Sacramento, Portugal
António Salgado, Portugal
Arnaud Scherberich, Switzerland
Romana Schirhagl, Netherlands
Günther Schlunck, Germany
Daniel Seitz, Germany
Ólafur Sigurjonsson, Iceland
Cyrill Slezak, United States
Martin Stoddart, Switzerland
Tang D. Teng,
Luc Téot, France
Andreas Traweger, Austria
Daphne Van der Heide, Switzerland
Jan Van Hest, Netherlands
Gerjo Van Osch, Netherlands
Shyni Varghese, United States
Giovanni Vozzi, Italy
Wenxin Wang, Ireland
Thomas Webster, United States
Bettina Weigelin, Germany
Carsten Werner, Germany
Britt Wildemann, Germany
Germar Wittke, Germany
Kenneth W. Witwer, United States
Michał Wszoła, Poland
Ozlem Yesil-Celiktas, Turkey
David Young, United Kingdom
Maximina Yun, Germany
Y. Shrike Zhang, United States

Arti Ahluwalia
Mohammad Alkhraisat
Andrea Barbero
Aleksandra Benko
Leonora Bużańska
Maria Chatzinikolaidou
Xanthippi Chatzistavrou
Valeria Chiono
Anna Chróścicka
Marta Alves Da Silva
Andrew Daly
Serena Danti
Laura De Laporte
Carmelo De Maria
Elena De-Juan-Pardo
Lucia Gemma Delogu
Anne Des Rieux
Sanjiv Dhingra
Ruslan I. Dmitriev
Rui M. A. Domingues
Timothy Douglas
Peter Dungel
Mirosława El Fray
Silvia Farè
Eric Farrell
Nicholas Forsyth
Giancarlo Forte
Debby Gawlitta
Iris Gerner
Cecilie Gjerde
Manuela E. Gomes
Gabriela Graziani
Thomas Groth
Debbie Guest
Alicia El Haj
Ales Hampl
Melanie Hart
Akon Higuchi
Veronika Hruschka
Joanna Idaszek
Gunil Im
Anna-Dimitra Kataki
Marta Klak
Aleksandra Klimczak
Zuzana Koci
Lisanne Laagland
Luminita Labusca
Irene Lara-Saez
Catherine Le Visage
Riccardo Levato
Andrea Lolli
Yi-tung Lu
Barbara Łukomska
Srinivas Madduri
Marcin Majka
Jos Malda
João Mano
Faleh Marino
Alexandra P. Marques
Petra Mela
Silvia Maria Mihăilă
Aline F. Miller
Farzaneh Moghtader
Michael Monaghan
Lorenzo Moroni
Carlos Mota
Kamal Mustafa
Nuno Neves
Beata Niemczyk-Soczynska
Sylvia Nürnberger
Fergal O'Brien
Mikolaj Ogrodnik
Dammy Olayanju
Julieta I. Paez
Elżbieta Pamuła
Abhay Pandit
Ioannis Papantoniou
Zygmunt Pojda
Giovanna Della Porta
Xiao-hua Qin
Heinz Redl
Rui L. Reis
Geoff Richards
Bernd Rolauffs
Laura Russo
Jakub Rybka
Joanna Sadowska
Alberto Saiani
Paweł Sajkiewicz
Manuel Salmeron-Sanchez
Arnaud Scherberich
Christina Schofield
Markus Schosserer
Daniel Seitz
Tiziano Serra
Heungsoo Shin
Y. Shrike Zhang
Paul Slezak
James E. Smay
Martin Stoddart
Yuto Takemoto
Jeremy Teo
Marianna Tryfonidou
Lizette Utomo
Gerjo van Osch
Sandra Van Vlierberghe
Paula Vena
Wenxin Wang
Lukasz Witek
Jacek K. Wychowaniec
Qian Xu
Ozlem Yesil-Celiktas
Marcy Zenobi-Wong
Dimitrios I. Zeugolis
Ewa Zuba-Surma

CONFERENCE VENUE

ICE Congress Centre
Address: Marii Konopnickiej 17
30-302 Kraków
http://icekrakow.pl/en
ICE is ~13.7 km away from Balice International Airport

CONFERENCE SESSION TIMES

Tuesday 28 June	9:00 – 18:30
Wednesday 29 June	9:00 – 18:30
Thursday 30 June	9:00 – 18:30
Friday 1 July	9:00 – 13:30

Please see the full conference programme for specific details.

EXHIBITION OPENING HOURS

Tuesday 28 June	9:00 – 18:30
Wednesday 29 June	9:00 – 18:30
Thursday 30 June	9:00 – 18:30
Friday 1 July	9:00 – 13:30

CONFERENCE REGISTRATION DESK

Monday 27 June	16:00 – 19:30
Tuesday 28 June	8:00 – 18:30
Wednesday 29 June	8:30 – 18:30
Thursday 30 June	8:30 – 18:30
Friday 1 July	8:30 – 12:30

WI-FI

Wi-Fi will be provided throughout the conference
SSiD: TERMIS-EU-2022
Password: Krakow22

PODIUM PRESENTATIONS

All podium presenters are requested to bring their presentation files (pdf or ppt format), using a USB stick, to the Speaker's Room (Level +3) the day before their talk. Please ensure that your videos run properly. No one will be allowed to present from their own computer. All speakers must keep to their allocated presentation time (e.g. Plenary Speakers: 60 min talk; Invited Speakers: 18 min talk + 2 min questions and answers; Other Speakers: 8 min talk + 2 min questions and answers). Sessions chairs are instructed to not allow speakers run over time.

POSTER PRESENTATIONS

The poster boards are ordered in numerical order. Please use your allocated poster board number. Posters will be displayed for the duration of the meeting. Organisers do not take responsibility for posters remaining at the end.

USE OF MOBILE PHONES

Use of mobile phones is not allowed in the lecture halls during sessions. Please be considerate of others by turning off your mobile phone.

NAME BADGE

Your name badge is your admission pass to TERMIS EU 2022 scientific sessions, exhibitions, coffee and lunch breaks, Welcome Reception, TERMIS Dinner. Without your name badge, admission will not be allowed. Accompanied persons who have not registered will be able to purchase tickets for the Welcome Reception, TERMIS Dinner on the date at the venue.

FOOD AND DRINK

Tea and coffee will be served throughout conference from various stations located on level 0, Level +1 and Level +2.
Delegate lunches (lunchboxes) will be served in these areas.

WELCOME RECEPTION

Welcome reception will be held on 28 June at the ICE Congress Centre (Level 0, Level +1) 20:00 -22:00. There will be finger food, wine and soft drinks served during Welcome Reception.

SYIS NIGHT

Forty Kleparz Music Club
Address: Kamienna 2-4
Date: Wednesday 29 June
Start time: 20:00
End time: 24:00
Getting there: on your own.

There will be finger food, wine and soft drinks served during SYIS night.

TERMIS DINNER

Stara Zajezdnia Restaurant
Address: Świętego Wawrzyńca 12
Date: Thursday 30 June
Start time: 20:00
End time: 23:30
Getting there: on your own.
There will be buffet menu, wine and beer served during the TERMIS Dinner.

INSURANCE

The conference organization does not take out insurance to cover any individual against accidents, thefts or other risks.

CURRENCY & MONEY EXCHANGE

The official currency of Poland is the Zloty (PLN). The exchange rate is approximately 1 EUR to 4.60 PLN (1 USD 4.70 PLN). Banks are open from Monday to Friday from 09:30 to 18:00. Exchange offices can be found all around Krakow and are clearly marked. ATMs are widely available around the city and shops accept major credit cards.

OFFICIAL LANGUAGE

Official language is Polish. English is widely spoken.

WEATHER

June average temperatures in Krakow in recent years: 15 to 30°C (62 to 77°F). It may rain.

LOCAL TIME

Local time in Krakow: summer time DST; UTC +2 (CEST).

ELECTRICITY

Electric power is 230 V, 50 Hz. Outlet plugshare C / E (2-pins: C is not grounded and E is grounded). Most of European countries use this socket standard (e.g. Germany, France, Belgium). It is compatible with C / F (C / E / Fare most common socket standards in Europe).
https://www.worldstandards.eu/electricity/plugs-andsockets/

FIRST AID

A first aid service will be available at the ICE at all times during the congress. Please note, however, no other medical service will be provided. Participants are expected to cover the costs of any medical expenses incurred in Poland. Therefore, it is highly recommended that participants have an insurance policy covering medical expenses in Poland and elsewhere during their travel. EU citizens should carry the European Health Insurance Card. Participants requesting an entry visa will also be required to submit proof of medical insurance.

MEDICAL SERVICES

Krakow has a good network of health centers (primary care services) and hospitals. In the case of an emergency, injury, sudden illness or deterioration to health, patients should either call an ambulance or go directly to a hospital, to the A&E department. In such cases, medical transport is free of charge. In hospital, you must present your valid European Health Insurance Card or a replacement certificate or insurance.

EMERGENCY PHONE NUMBERS

Dial without any area code:
112 General emergency number
997 Police
998 Fire Brigade
999 Ambulance
Note: Emergency calls are toll-free.

SMOKING

Smoking is prohibited at the conference venue or in any other public indoor establishment and bus or tram stops.

TAXI

In order to avoid unpleasant surprises, we recommend only using licensed taxi companies. Such taxis should have a visible

price-list, a taximeter and a company logo with a phone number. Taxis can be ordered by phone, some of them online or at a taxicab stand. You will pay approx. 8.00 zloty (1.80 euro) upfront charge and 3.00 zloty (0.64 euro) / km.

PUBLIC TRANSPORTATION:

Public transportation (trams and buses in Kraków) for TERMIS 2022 participants is free of charge from 28.06 till 1.07.2022.
You will receive hologram sticker during registration, that must be stuck on the badge. **Remember, that only badge with hologram entitles you to use free public transportation** (except transfer to the airport). The current timetable is available at: https://rozklady.mpk.krakow.pl
We recommend using the website: https://jakdojade.pl/krakow

COVID -19 INFORMATION AND RECOMMENDATIONS

Since 28 March, 2022, pursuant to the provisions of the Regulation of the Council of Ministers of March 25, 2022 on the establishment of certain restrictions and limitations in connection with an epidemic (Journal of Laws, item 673), all travel restrictions have been lifted, what means that there is no longer an obligation to:

- show vaccination certificates when crossing the border,
- test for SARS-CoV-2,
- be quarantined,
- wear masks (the exception applies to medical entities and pharmacies).

The conference organizer provides disinfectants and masks in the conference venue.

LONG-TERM LEGACY -TERMIS EU 2022 PLANT THE TREE IN “CONGRESS AVENUE

“Congress Avenue” is a series of events during which representatives of the City of Krakow, the ICE
Congress Centre and Congress Organizing Committees symbolically plant tree at Monte Cassino Street to emphasize the real concern for the environment, the longest green trees, absorbing dust from the air, are planted.
The tree commemorates an important event hosted by the city – the first of 30 planned trees was planted after 41st session of the UNESCO World Heritage Committee in July 2017. A sign informing about the congress during which the tree was planted is placed next to each tree.
Representatives of the meetings industry emphasize increasing importance to ecology and the sustainable development in the context of the organization the congresses and conferences. This is also a symbol of a positive impact that congresses have on the city and its residents. Congress Avenue allows to draw attention among media and show the approach of Congress organizers and Krakow city to the subject of sustainable development as well as the long-term legacy that can be generated by scientific meetings.

PROJECT OBJECTIVES:

a. Presentation of the constant care of the City of Krakow, ICE Kraków and event organizers for the natural environment in the immediate vicinity of the Congress Center, in the city center.
b. Exposition of direct, positive impact of events implemented at ICE Kraków - urban investment on the city’s environment and a symbol of positive impact of the meetings on the city
c. Consolidating the image of the City of Krakow and ICE Kraków as an excellent, strong, modern and ecological destination for business meetings.
d. Increasing the competitiveness of ICE Kraków - participation in the project is also an image gain for the event organizer.
e. Emphasizing the importance of events organized in Krakow.

(Latin: Cracovia, French: Cracovie, German: Krakau, Jidish: קראָקע, "Kroke", also Cracow or Kraków). Kraków is a city with surface rights, located in southern Poland on the Vistula River, as the second largest city in Poland both with regard to the population and surface area.

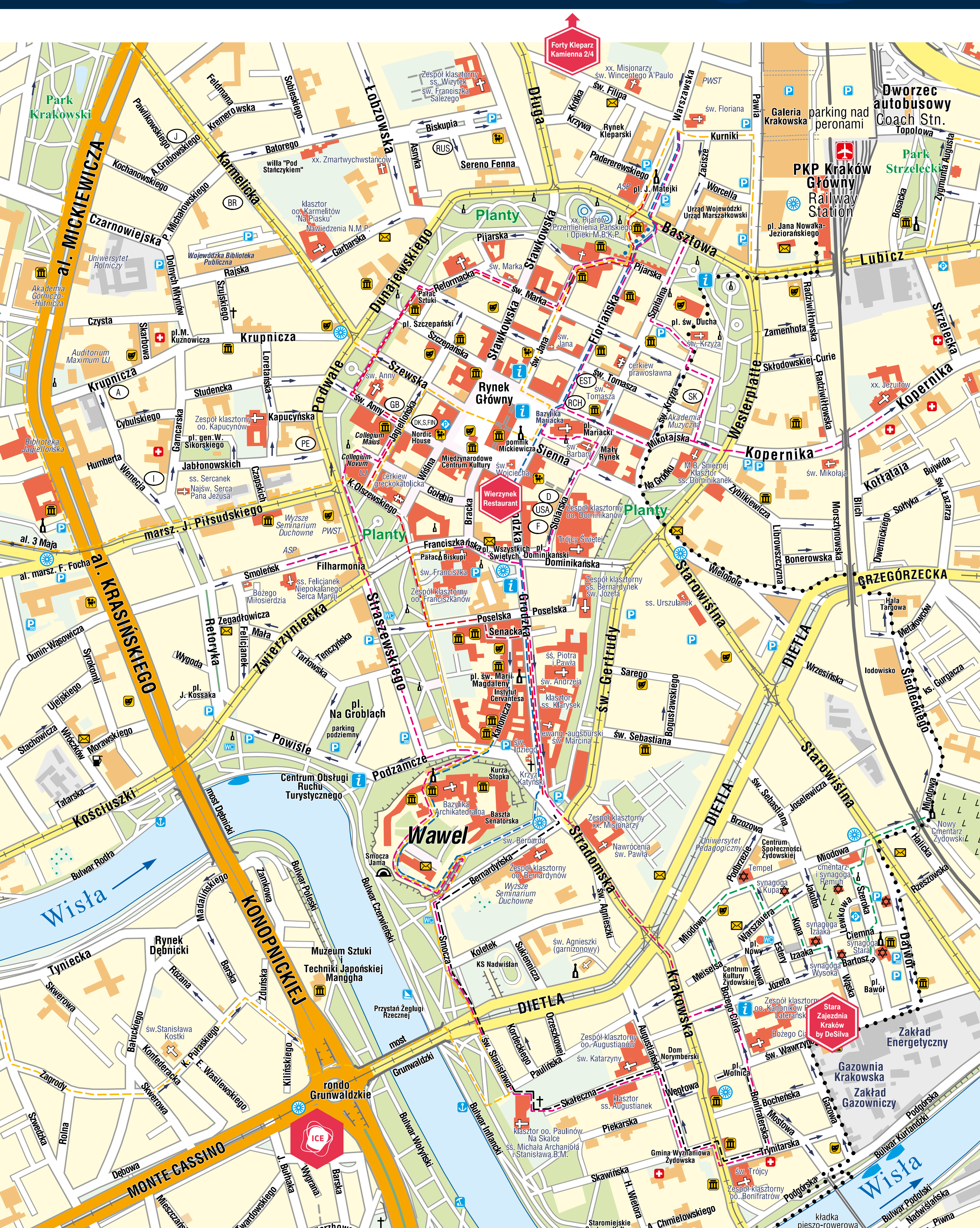
It is a former capital of Poland, Royal Capital City and necropolis of Polish kings, as well as the capital of Małopolska Region. The ancient, royal city of Kraków is a unique symbol of Polish national identity. Enchantingly picturesque, rich in relics of all epochs, it represents the thousand-year-long history of the Polish nation.

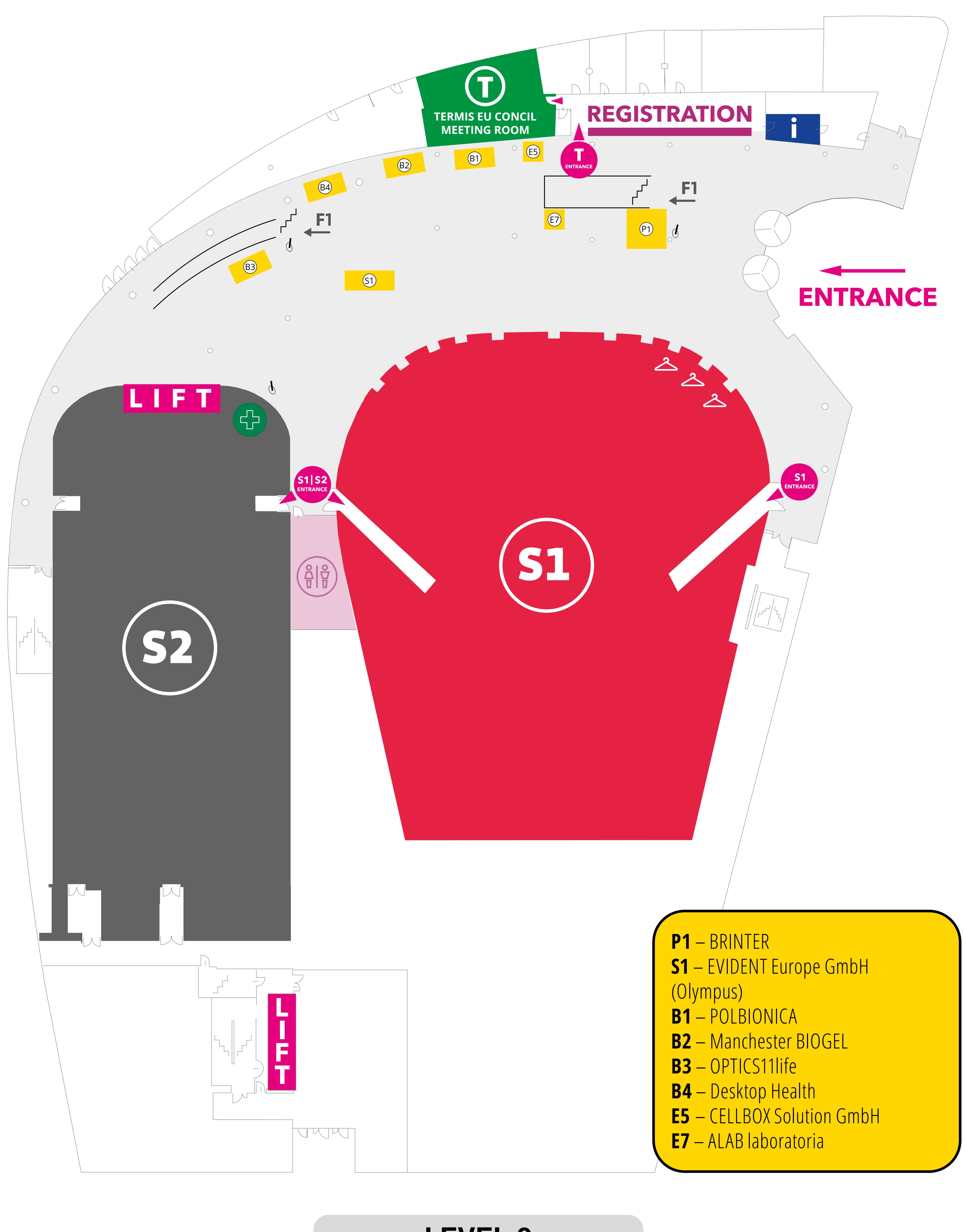
In Kraków you can admire many different styles of architecture, unique Romanesque objects, monumental Gothic edifices, and masterpieces by some of the most outstanding architects of the Renaissance and Baroque period. Kraków has always been a centre of Polish culture and science.

Kraków is one of the best recognizable cities in this part of Europe, a favourite destination of individual and group trips. The city attracts visitors with its legend: a treasure trove of Polish heritage, European Capital of Culture, City of Literature UNESCO, a city full of events and unique collections, an organizer of excellent festivals. Currently, Kraków is the artistic and intellectual centre of southern Poland, and one of the most popular tourist spot in Europe.

Kraków's main Market Square – the largest mediaeval square in Europe (200x200 meters) – has always been the heart of the city, vibrant with life throughout the year. It is a favorite meeting place for Cracovians, students, tourist, and businesspeople. The 47 tenement buildings surrounding the square house numerous cafés, restaurants, pubs, galleries, shops, bookstores, and museums.

Wawel and the Kraków Old Town, alongside old Jewish district Kazimierz, were selected for the original UNESCO World Heritage List, created in 1978. Other locations include the Salt Mine in Wieliczka, the oldest active salt mine in the world, located 12 kilometers south of Kraków.


Forty Kleparz
Kamienna 2/4
Park Krakowski
al. MICKIEWICZA
Czarnowiejska
Karmelicka
Łobzowska
Długa
Warszawska
Pawia
Galeria Krakowska
parking nad peronami
Dworzec autobusowy Coach Stn.
Topolowa
PKP Kraków Główny Railway Station
Park Strzelecki
Basztowa
Lubicz
Planty
Dunajewskiego
Krupnicza
Floriańska
Sławkowska
Szczepańska
Szewska
Rynek Główny
Westerplatte
Kopernika
Podwale
marsz. J. Piłsudskiego
al. 3 Maja
al. KRASIŃSKIEGO
Zwierzyniecka
Straszewskiego
Franciszkańska
Dominikańska
Grodzka
Sienna
Starowiślna
GRZEGÓRZECKA
DIETLA
Wierzynek Restaurant
Filharmonia
pl. Na Groblach
Powiśle
Podzamcze
Centrum Obsługi Ruchu Turystycznego
Wawel
Bazylika Archikatedralna
Kościuszki
Wisła
Bulwar Rodła
św. Gertrudy
Stradomska
Krakowska
KONOPNICKIEJ
Muzeum Sztuki i Techniki Japońskiej Manggha
Rynek Dębnicki
Tyniecka
rondo Grunwaldzkie
ICE
MONTE CASSINO
Stara Zajezdnia Kraków by DeSilva
Gazownia Krakowska
Zakład Energetyczny
Zakład Gazowniczy
Skałeczna
Piekarska
Skawińska
Trynitarska
Józefa
Miodowa
Szeroka
Dajwór
Bulwar Kurlandzki
kładka pieszo-rowerowa


TERMIS EU CONCIL MEETING ROOM
REGISTRATION
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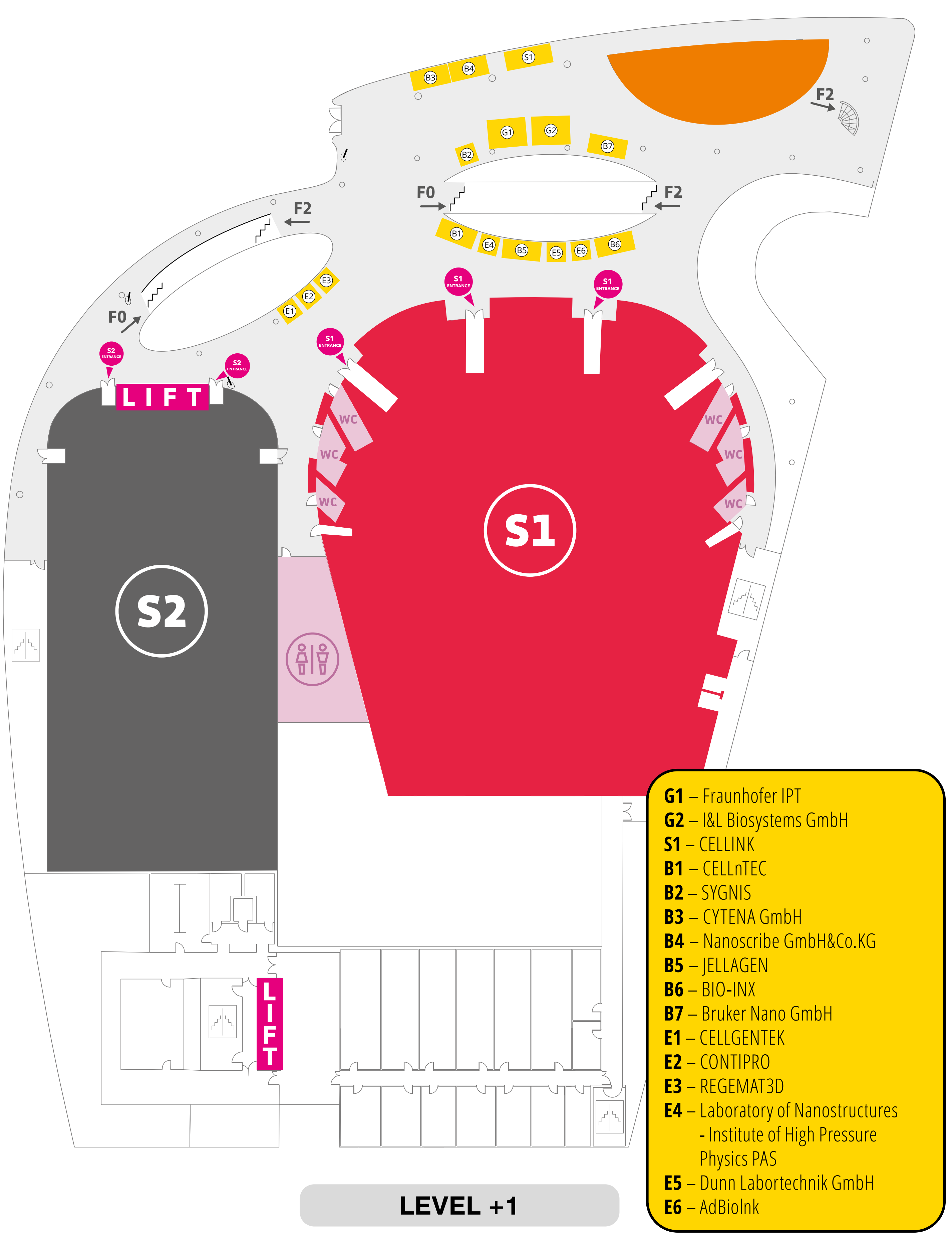
T ENTRANCE
F1
F1
ENTRANCE
LIFT
S1|S2 ENTRANCE
S1 ENTRANCE
S1
S2
LIFT
B1
B2
B3
B4
E5
E7
P1
S1
P1 – BRINTER
S1 – EVIDENT Europe GmbH (Olympus)
B1 – POLBIONICA
B2 – Manchester BIOGEL
B3 – OPTICS11life
B4 – Desktop Health
E5 – CELLBOX Solution GmbH
E7 – ALAB laboratoria
LEVEL 0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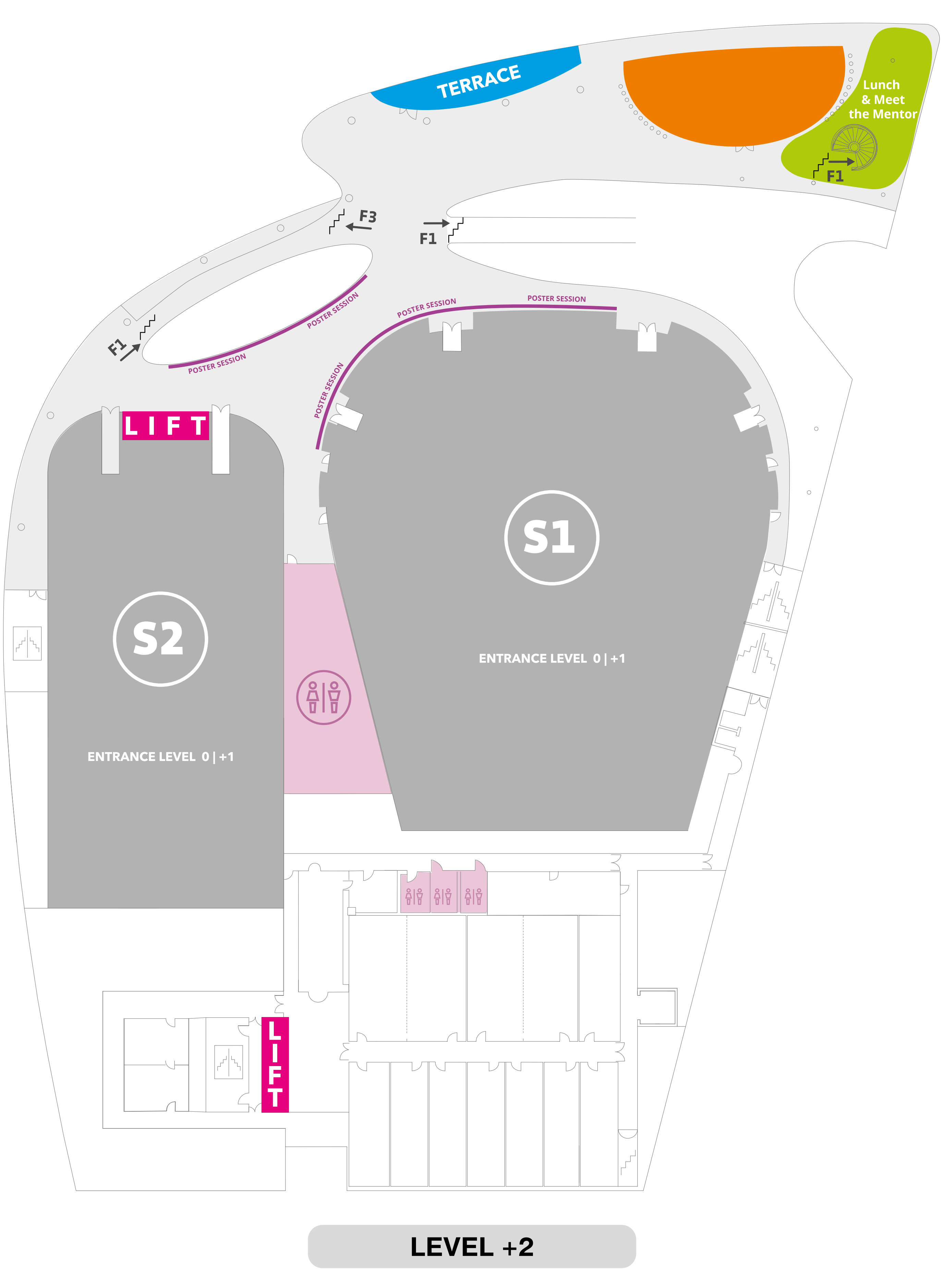
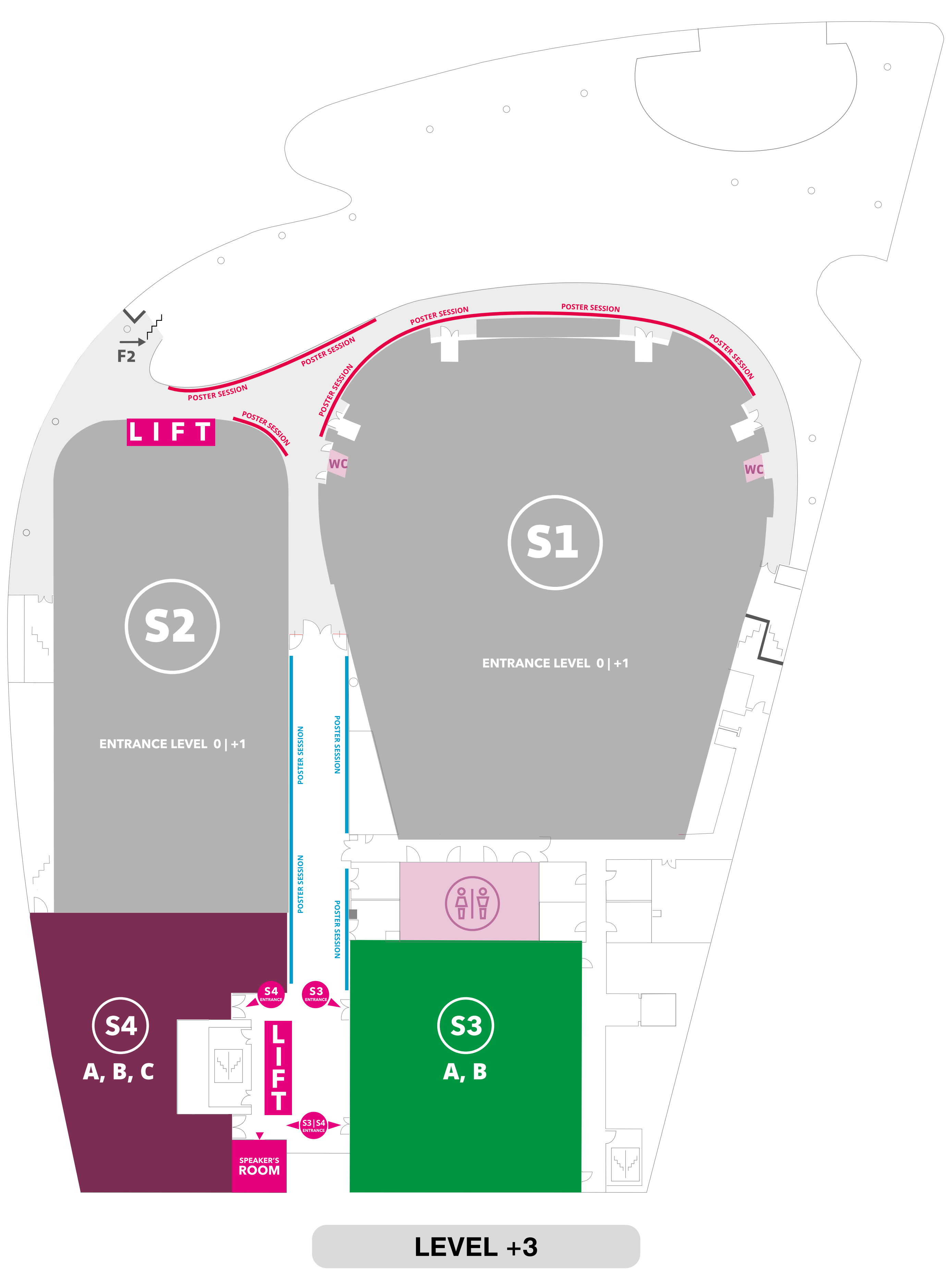
LEVEL +1
G1 – Fraunhofer IPT
G2 – I&L Biosystems GmbH
S1 – CELLINK
B1 – CELLnTEC
B2 – SYGNIS
B3 – CYTENA GmbH
B4 – Nanoscribe GmbH&Co.KG
B5 – JELLAGEN
B6 – BIO-INX
B7 – Bruker Nano GmbH
E1 – CELLGENTEK
E2 – CONTIPRO
E3 – REGEMAT3D
E4 – Laboratory of Nanostructures - Institute of High Pressure Physics PAS
E5 – Dunn Labortechnik GmbH
E6 – AdBioInk



LEVEL +2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S2
ENTRANCE LEVEL 0 | +1
POSTER SESSION
POSTER SESSION
POSTER SESSION
POSTER SESSION
S4 ENTRANCE
S3 ENTRANCE
S4
A, B, C
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S3 | S4 ENTRANCE
SPEAKER'S ROOM
S3
A, B
LEVEL +3

P1	**BRINTER** brinter.com
S1	**EVIDENT Europe GmbH (Olympus)** olympus.com
B1	**POLBIONICA** www.polbionica.com
B2	**Manchester BIOGEL** manchesterbiogel.com
B3	**OPTICS11life** optics11life.com
B4	**Desktop Health** desktophealth.com
E5	**CELLBOX Solution GmbH** www.cellbox-solutions.com
E7	**ALAB laboratoria** www.alablaboratoria.pl

Stand	Exhibitor	Website
G1	**Fraunhofer IPT**	www.ipt.fraunhofer.de
G2	**I&L Biosystems GmbH**	www.il-biosystems.com
S1	**CELLINK**	www.cellink.com
B1	**CELLnTEC**	www.cellntec.com
B2	**SYGNIS**	sygnis.pl
B3	**CYTENA GmbH**	www.cytena.com
B4	**Nanoscribe GmbH&Co.KG**	www.nanoscribe.com

B5

JELLAGEN

www.jellagen.co.uk

B6

BIO-INX

www.xpect-inx.com

B7

Bruker Nano GmbH

www.jpk.com, www.bruker.com/bioafm

E1

CELLGENTEK

www.bioimagingsystem.com

E2

CONTIPRO

contipro.com

E3

REGEMAT3D

www.regemat3d.com

E4

Laboratory of Nanostructures - Institute of High Pressure Physics PAS

labnano.pl

Dunn Labortechnik GmbH

E5

www.dunnlab.de

AdBioInk

E6

adbioink.com

Time/date	Monday 27.06.2022		Tuesday 28.06.2022	Wednesday	Thursday 30.06.2022	Friday 01.07.2022
08:30			Registration		Registration	Registration
09:00 – 10:00			Opening Session		enary Session	Plenary Session
10:30			Coffee break & poster		e break & poster	Coffee break
11:00 – 12:00			Parallel Symposia		allel Symposia	Parallel Symposia
12:30 – 13:00	Workshop registration		Lunch	Lu	& Meet the Mentor	Closing Session & Awards
13:30 – 14:30	Pre-conference Workshop		Parallel Symposia	Parallel Symposia	Parallel Symposia	
15:00			Coffee break & poster	Coffee break & poster	Coffee break & poster	
15:30 – 16:30		Conference Registration	Parallel Symposia	Parallel Symposia	Parallel Symposia	
17:00			Coffee break & poster	Coffee break & poster	Coffee break & poster	
17:30 – 18:00			Awards Session	Plenary Session	FTERM Panel Discussion	
18:30 – 19:00			General Assembly	SYIS Career Panel	SYIS Green lab Panel Discussion	
19:30						
20:00 – 22:00			Welcome reception	SYIS Night	TERMIS Dinner	

Time	Room S1	Room S2	Room S3 A	Room S3 B	Room S4 A	Room S4 B	Room S4 C
Tuesday, 28 June 2022							
09:00 - 10:30	Opening Session						
10:30 - 11:00	Coffee break & poster						
11:00 - 12:30	S01 3D in vitro tissue-engineered cancer/disease models – Session I	S13-1 Biofunctionalized surfaces for cellular and tissue engineering	S07-1 Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration	S49 Novel strategies to assess cellular response to biomaterials	S08 Antimicrobial biomaterials for bone regeneration		
12:30 - 13:30	Lunch break						
13:30 - 15:00	S02 3D in vitro tissue-engineered cancer/disease models – Session II	S16-1 Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential	S06 Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective	S17 Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility	S19 Biomimetic Approaches to Cardiovascular Regeneration: how and why?	S09 Biobanking - indispensable support for the development of regenerative medicine	S25+S64 Cellular senescence in tissue damage and regeneration + Understanding and preventing earl'y inflammatory events that lead to development of osteoarthritis
15:00 - 15:30	Coffee break & poster						
15:30 - 17:00	S15-1 Biologically inspired and Engineered disease models	S16-2 Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential	S07-2 Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration	S03+S33 3D printing of bionic organs – how far are we from clinical application? + From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine	S68 Human brain organoids versus assembloids approach for neurodevelopmental studies	S04 3D Writing Within Suspension Media for Tissue Engineering and In Vitro Modeling	S22 Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine
17:00 - 17:30	Coffee break & poster						
17:30 - 19:00	Awards Session						
19:00 - 20:00	General Assembly						
20:00 - 22:00	Welcome reception						
Wednesday, 29 June 2022							
09:00 - 10:00	P1 Plenary Session: Cartilage regeneration: the challenges of regenerating a "simple" non-vascularised tissue						
10:00 - 10:30	Debate 1: Regeneration of human joints						
10:30 - 11:00	Coffee break & poster						
11:00 - 12:30	S10-1 Biofabricated Tissues and Organs for Clinical Impac	S43-1 Multifunctional biomaterials supporting bone regeneration	S13-2 Biofunctionalized surfaces for cellular and tissue engineering	S12 Biofabrication with light-based technologies and high-definition printing	S48 Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy	S62 Tissue regeneration by integration of bioinspired materials	S28 Emerging and future technologies for peripheral nerve regeneration
12:30 - 13:30	Lunch & Meet the Mentor						
13:30 - 15:00	S24 Cell-rich constructs for tissue engineering	S43-2 Multifunctional biomaterials supporting bone regeneration	S31 Extracellular vesicles – next generation tool for diagnostics and regenerative medicine	S38 Injectable biomaterials for cell-instructive matrix cues	S37 Human Organoids for Musculoskeletal Tissues	S20 Biomimetic in vitro models for bone regeneration and cancer pathologies	S30 European regional platforms for TERM - Update
15:00 - 15:30	Coffee break & poster						
15:30 - 17:00	S15-2 Biologically inspired and Engineered disease models	S47 New insights underlying mesenchymal stem cell-mediated bone regeneration	S41 Mesenchymal Stem / Stromal Cells - from basic research through clinical studies to registered products	S11 Biofabrication using extrinsic fields	S23+S31+S32 Can we bioengineer tissues using artificial cells? + Extracellular vesicles – next generation tool for diagnostics and regenerative medicine + Extracellular vesicles for soft tissue repair	S27+S56 Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses + Skin wound healing in 2022: where basic science meets clinical needs	
17:00 - 17:30	Coffee break & poster						
17:30 - 18:30	P2 Plenary Session: Engineering in Precision Medicine						
18:30 - 19:30		SYIS Career Panel					
20:00 - 22:00	SYIS Night						
Thursday, 30 June 2022							
09:00 - 10:00	P3 Plenary Session: Bioprinting 3D vascularized tissue flaps						
10:00 - 10:30	Debate 2: Beyond the promise of Biofabrication: what needs to be done to bring biofabricated substitutes to the clinic?						
10:30 - 11:00	Coffee break & poster						
11:00 - 12:30	S10-2 Biofabricated Tissues and Organs for Clinical Impact	S39 Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering	S05 Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice	S52 Perspectives For Future Innovation in Tendon repair (P4 FIT)	S55 REMODELing the Future: next generation of organoid models for biomedicine	S60 Tissue engineering and regenerative medicine in Czech Republic	S66 Wanted: Dead or Alive? Quantitative microscopy of spheroid and organoid tissues
12:30 - 13:30	Lunch & Meet the Mentor						
13:30 - 15:00	S65-1 Vascularization for Tissue Engineering and Regenerative Medicine	S57 Supramolecular synthetic scaffolds: from concept to design and application	S59+S18 The role of multifunctional nanomaterials in new tissue regeneration strategies + Biomedical applications of MXene based next generation nanomaterials	S63 Towards automated technologies for organoid-based tissue biomanufacturing	S67 We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration	S53 Prospects and Challenges in Biological Therapies for Tendon Regeneration	S26 Combined Korea-EU Symposium: "Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells"
15:00 - 15:30	Coffee break & poster						
15:30 - 17:00	S65-2 Vascularization for Tissue Engineering and Regenerative Medicine	S40 Injectable scaffolds in tissue engineering	S45 Nature bioinspired biomaterials and strategies for TERM	S42 Microphysiological models as powerful preclinical tools	S51+S29 Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine + Engineered viscoelasticity in cell and tissue engineering	S21+S44 Biophysical Therapies - External energy to push internal regeneration + Nano Magnetic platforms - an attractive opportunity for advancing TERM products to the clinic	S58 TERMIS-EU SYIS and yESAO joint symposium
17:00 - 17:30	Coffee break & poster						
17:30 - 18:30	FTERM Panel Discussion						
18:30 - 19:30		SYIS-GLP SYIS Green Lab Panel Discussion					
20:00 - 22:00	TERMIS Dinner						
Friday, 1 July 2022							
09:00 - 10:00	P4 Plenary Session: Commentatio historica et philologica Perspectives and Challenges in Regenerative Medicine						
10:00 - 10:30	Debate 3: Perspectives and Challenges of Tissue engineering and Regenerative Medicine						
15:30 - 17:00	Coffee break						
11:00 - 12:30	S46 New developments of regenerative and tissue modeling products	S54+S14 Regulation of cell phenotype in osteochondral tissues: towards RNA therapy for bone and cartilage repair + Biological testing of 3D-printed biomaterials towards updated norms	S35+S36 Giving meaning to early tissue damage responses in regeneration + Glycomodulation Approaches in Tissue Engineering	S50 One health, one medicine: What Veterinary regenerative medicine can teach us	S34 Advanced therapy approaches in tissue engineering	S61 Tissue Engineering in Microgravity for Health in Space and on Earth	
12:30 - 13:30	Closing Session and Awards						

Tuesday, 28th June 2022			
no.	**title**	**room**	**time**
OP-S	Opening Session	S1	(09:00 - 10:30)
S01	3D in vitro tissue-engineered cancer/disease models – Session I	S1	(11:00 - 12:30)
S13-1	Biofunctionalized surfaces for cellular and tissue engineering	S2	(11:00 - 12:30)
S07-1	Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration	S3 A	(11:00 - 12:30)
S49	Novel strategies to assess cellular response to biomaterials	S3 B	(11:00 - 12:30)
S08	Antimicrobial biomaterials for bone regeneration	S4 A	(11:00 - 12:30)
S02	3D in vitro tissue-engineered cancer/disease models – Session II	S1	(13:30 - 15:00)
S16-1	Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential	S2	(13:30 - 15:00)
S06	Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective	S3 A	(13:30 - 15:00)
S17	Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility	S3 B	(13:30 - 15:00)
S19	Biomimetic Approaches to Cardiovascular Regeneration: how and why?	S4 A	(13:30 - 15:00)
S09	Biobanking - indispensable support for the development of regenerative medicine	S4 B	(13:30 - 15:00)
S25 +S64	Cellular senescence in tissue damage and regeneration + Understanding and preventing early inflammatory events that lead to development of osteoarthritis	S4 C	(13:30 - 15:00)
S15-1	Biologically inspired and Engineered disease models	S1	(15:30 - 17:00)
S16-2	Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential	S2	(15:30 - 17:00)
S07-2	Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration	S3 A	(15:30 - 17:00)
S03 +S33	3D printing of bionic organs – how far are we from clinical application? + From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine	S3 B	(15:30 - 17:00)
S68	Human brain organoids versus assembloids approach for neurodevelopmental studies	S4 A	(15:30 - 17:00)
S04	3D Writing Within Suspension Media for Tissue Engineering and In Vitro Modeling	S4 B	(15:30 - 17:00)
S22	Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine	S4 C	(15:30 - 17:00)
AW-S	Awards Session	S1	(17:30 - 19:00)
GA	General Assembly	S1	(19:00 - 20:00)

Wednesday, 29th June 2022			
no.	**title**	**room**	**time**
P1	Plenary Session: Cartilage regeneration: the challenges of regenerating a "simple" non-vascularised tissue	S1	(09:00 - 10:00)
Deb 1	Debate 1: Regeneration of human joints	S1	(10:00 - 10:30)
S10-1	Biofabricated Tissues and Organs for Clinical Impact	S1	(11:00 - 12:30)
S43-1	Multifunctional biomaterials supporting bone regeneration	S2	(11:00 - 12:30)
S13-2	Biofunctionalized surfaces for cellular and tissue engineering	S3 A	(11:00 - 12:30)
S12	Biofabrication with light-based technologies and high-definition printing	S3 B	(11:00 - 12:30)
S48	Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy	S4 A	(11:00 - 12:30)
S62	Tissue regeneration by integration of bioinspired materials	S4 B	(11:00 - 12:30)
S28	Emerging and future technologies for peripheral nerve regeneration	S4 C	(11:00 - 12:30)
S24	Cell-rich constructs for tissue engineering	S1	(13:30 - 15:00)
S43-2	Multifunctional biomaterials supporting bone regeneration	S2	(13:30 - 15:00)
S31	Extracellular vesicles – next generation tool for diagnostics and regenerative medicine	S3 A	(13:30 - 15:00)
S38	Injectable biomaterials for cell-instructive matrix cues	S3 B	(13:30 - 15:00)
S37	Human Organoids for Musculoskeletal Tissues	S4 A	(13:30 - 15:00)
S20	Biomimetic in vitro models for bone regeneration and cancer pathologies	S4 B	(13:30 - 15:00)
S30	European regional platforms for TERM - Update	S4 C	(13:30 - 15:00)
S15-2	Biologically inspired and Engineered disease models	S1	(15:30 - 17:00)
S47	New insights underlying mesenchymal stem cell-mediated bone regeneration	S2	(15:30 - 17:00)
S41	Mesenchymal Stem / Stromal Cells - from basic research through clinical studies to registered products	S3 A	(15:30 - 17:00)
S11	Biofabrication using extrinsic fields	S3 B	(15:30 - 17:00)
S23 +S31 +S32	Can we bioengineer tissues using artificial cells? + Extracellular vesicles – next generation tool for diagnostics and regenerative medicine + Extracellular vesicles for soft tissue repair	S4 A	(15:30 - 17:00)
S27 +S56	Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses + Skin wound healing in 2022: where basic science meets clinical needs	S4 B	(15:30 - 17:00)
P2	Plenary Session: Engineering in Precision Medicine	S1	(17:30 - 18:30)
SYIS-CP	SYIS Career Panel	S2	(18:30 - 19:30)

Thursday, 30th June 2022			
no.	**title**	**room**	**time**
P3	Plenary Session: Bioprinting 3D vascularized tissue flaps	S1	(09:00 - 10:00)
Deb 2	Debate 2: Beyond the promise of Biofabrication: what needs to be done to bring biofabricated substitutes to the clinic?	S1	(10:00 - 10:30)
S10-2	Biofabricated Tissues and Organs for Clinical Impact	S1	(11:00 - 12:30)
S39	Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering	S2	(11:00 - 12:30)
S05	Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice	S3 A	(11:00 - 12:30)
S52	Perspectives For Future Innovation in Tendon repair (P4 FIT)	S3 B	(11:00 - 12:30)
S55	REMODELing the Future: next generation of organoid models for biomedicine	S4 A	(11:00 - 12:30)
S60	Tissue engineering and regenerative medicine in Czech Republic	S4 B	(11:00 - 12:30)
S66	Wanted: Dead or Alive? Quantitative microscopy of spheroid and organoid tissues	S4 C	(11:00 - 12:30)
S65-1	Vascularization for Tissue Engineering and Regenerative Medicine	S1	(13:30 - 15:00)
S57	Supramolecular synthetic scaffolds: from concept to design and application	S2	(13:30 - 15:00)
S59 +S18	The role of multifunctional nanomaterials in new tissue regeneration strategies + Biomedical applications of MXene based next generation nanomaterials	S3 A	(13:30 - 15:00)
S63	Towards automated technologies for organoid-based tissue biomanufacturing	S3 B	(13:30 - 15:00)
S67	We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration	S4 A	(13:30 - 15:00)
S53	Prospects and Challenges in Biological Therapies for Tendon Regeneration	S4 B	(13:30 - 15:00)
S26	Combined Korea-EU Symposium: "Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells"	S4 C	(13:30 - 15:00)
S65-2	Vascularization for Tissue Engineering and Regenerative Medicine	S1	(15:30 - 17:00)
S40	Injectable scaffolds in tissue engineering	S2	(15:30 - 17:00)
S45	Nature bioinspired biomaterials and strategies for TERM	S3 A	(15:30 - 17:00)
S42	Microphysiological models as powerful preclinical tools	S3 B	(15:30 - 17:00)
S51 +S29	Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine + Engineered viscoelasticity in cell and tissue engineering	S4 A	(15:30 - 17:00)
S21 +S44	Biophysical Therapies - External energy to push internal regeneration + Nano Magnetic platforms - an attractive opportunity for advancing TERM products to the clinic	S4 B	(15:30 - 17:00)
S58	TERMIS-EU SYIS and yESAO joint symposium	S4 C	(15:30 - 17:00)
FTERM	FTERM Panel Discussion	S1	(17:30 - 18:30)
SYIS-GLP	SYIS Green Lab Panel Discussion	S2	(18:30 - 19:30)

Friday, 1st July 2022			
no.	**title**	**room**	**time**
P4	Plenary Session: Commentatio historica et philologica Perspectives and Challenges in Regenerative Medicine	S1	(09:00 - 10:00)
Deb 3	Debate 3: Perspectives and Challenges of Tissue engineering and Regenerative Medicine	S1	(10:00 - 10:30)
S46	New developments of regenerative and tissue modeling products	S1	(11:00 - 12:30)
S54 +S14	Regulation of cell phenotype in osteochondral tissues: towards RNA therapy for bone and cartilage repair + Biological testing of 3D-printed biomaterials towards updated norms	S2	(11:00 - 12:30)
S35 +S36	Giving meaning to early tissue damage responses in regeneration + Glycomodulation Approaches in Tissue Engineering	S3 A	(11:00 - 12:30)
S50	One health, one medicine: What Veterinary regenerative medicine can teach us	S3 B	(11:00 - 12:30)
S34	Advanced therapy approaches in tissue engineering	S4 A	(11:00 - 12:30)
S61	Tissue Engineering in Microgravity for Health in Space and on Earth	S4 B	(11:00 - 12:30)
CL-S&A	Closing Session and Awards	S1	(12:30 - 13:30)

Tuesday, 28 June 2022

Opening Session - Room: S1 (09:00 - 10:30)

time	title	presenter
09:00	Welcome speeches (30 minutes)	
09:30	Performance Art (15 minutes)	
09:45	The evolution of reconstructive surgery – team experience of Department od Oncological and Reconstructive Surgery National Research Institute of Oncology	MACIEJEWSKI, Adam

S01 3D in vitro tissue-engineered cancer/disease models – Session I - Room: S1 (11:00 - 12:30)

-Conveners: Anna-Dimitra Kataki; Silvia Farè

time	title	presenter
11:00	Bioengineered platform to study immune-cancer cell interactions ex vivo	VARGHESE, Shyni
11:20	Modelling breast-to-bone metastatic mechanisms via microfluidic biofabrication	CIDONIO, Gianluca
11:30	Induction of branching morphogenesis in cholangiocarcinoma organoids in vitro improves similarity with the original tumor for enhanced personalized medicine applications	VAN TIENDEREN, Gilles
11:40	A TUMOUR MICROENVIRONMENT MODEL FOR PANCREATIC CANCER	KAST, Verena
11:50	An In vitro Vascularised Liver Organotypic Model for the Testing of Nanomedicines	SANTIN, Matteo
12:00	A Systematic Comparative Assessment of the Response of Ovarian Cancer Cells to Cisplatin in 3D Models of Various Structural and Biochemical Configurations	KATAKI, Anna-Dimitra
12:10	HARNESSING PREDICTIVE TOXICOLOGY WITH A MINIATURIZED MODULAR GASTROINTESTINAL PLATFORM	NETO, Mafalda D.
12:20	Collagen-nanocellulose forms a matrix of controllable stiffness to mimic the pancreatic tumour microenvironment	CURVELLO, Rodrigo

S49 Novel strategies to assess cellular response to biomaterials - Room: S3 B (11:00 - 12:30)

-Conveners: Carmelo De Maria; Julieta I. Paez

time	title	presenter
11:00	Quantum Sensing for measuring free radical generation in living cells	SCHIRHAGL, Romana
11:20	A NEW SEMI-ORTHOTOPIC BONE DEFECT MODEL FOR CELL AND BIOMATERIAL TESTING IN REGENERATIVE MEDICINE	FARRELL, Eric
11:30	Mechanotransduction and reshaping at the nuclear envelope: investigating the Lamin A/C-SUN1 interaction	DONNALOJA, Francesca
11:40	IMMUNE PERFUSION IN CUSTOM BIOREACTORS FOR THE STUDY OF THE EXTRACELLULAR MATRIX-IMMUNE CELL CROSSTALK IN LIVER FIBROSIS	URBANI, Luca

11:50	GRAPHENE OXIDE PROMOTES EPITHELIAL MESENCHYMAL TRANSITION IN OVINE AMNIOTIC EPITHELIAL STEM CELLS AFFECTING THEIR IMMUNOMODULATORY PROPERTIES	CITERONI, Maria Rita
12:00	PARTICLE SIZE IN FREE-PACKED GRANULAR SYSTEMS INFLUENCE CELL RESPONSE	CUNHA, Ana F.
12:10	ELECTROACTIVE MATERIALS GOVERN CELL BEHAVIOR THROUGH THEIR EFFECT ON PROTEIN DEPOSITION	MARTIN-IGLESIAS, Sara
12:20	EVALUATION OF TISSUE INTEGRATION AND ANGIOGENESIS OF 3D PRINTED POROUS SCAFFOLDS USING A NON-DESTRUCTIVE MICROCT APPROACH	DIAZ-GOMEZ, Luis

S07-1 Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration - Room: S3 A (11:00 - 12:30)

-Conveners: Valeria Chiono; Michael Monaghan

time	title	presenter
11:00	Lessons learned on how (not to) build a heart	PASQUALINI, Francesco
11:20	MECHANICAL AND TOPOLOGICAL CUES TO ENHANCE DE NOVO EXTRACELLULAR MATRIX ELABORATION IN ELASTOMERIC SCAFFOLD MODELS.	D'AMORE, Antonio
11:40	Convergency of dual extrusion bioprinting and melt electrowriting allows for vascularized cardiac patch fabrication	AINSWORTH, Madison J.
11:50	Allogeneic stem cells and immunomodulatory biomaterials for cardiac tissue engineering	DHINGRA, Sanjiv
12:00	A Micro-Precision Electro Array (µPEA) platform integrated within a mechanically active heart-on-chip for modelling Dilated Cardiomyopathy	LOZANO-JUAN, Ferran
12:10	CARDIAC TISSUE-LIKE 3D MICROENVIRONMENT ENHANCES THE DIRECT REPROGRAMMING PATH OF HUMAN FIBROBLASTS INTO INDUCED CARDIOMYOCYTES BY MICRORNAS	PAOLETTI, Camilla
12:20	TISSUE ENGINEERED CARDIAC PATCHES FOR THE TREATMENT OF POST-MI HEART FAILURE USING NATURAL POLYMERS AND HUMAN IPSC-DERIVED CELLS	FRICKER, Annabelle

S08 Antimicrobial biomaterials for bone regeneration - Room: S4 A (11:00 - 12:30)

-Conveners: Fergal O'Brien; Joanna Sadowska

time	title	presenter
11:00	Bioactive glass based approaches for antibacterial bone regeneration	BOCCACCINI, Aldo
11:20	POLYHYDROXYALKANOATE/BIOACTIVE GLASS COMPOSITE SCAFFOLDS WITH ANTIMICROBIAL PROPERTIES FOR BONE TISSUE ENGINEERING APPLICATIONS	MELE, Andrea
11:30	Alpha Tocopherol, Alpha-tocopheryl Posphate and GN-2-Npm9, molecules for the modification of chemically treated Ti6Al4V alloy surfaces for antibacterial and anti-inflammatory purposes.	GAMNA, Francesca
11:40	BIOACTIVE GLASSES WITH ANTIBACTERIAL PROPERTIES FOR BONE TISSUE REGENERATION	ARANGO-OSPINA, Marcela
11:50	Drop on demand: A new method to develop antimicrobial coatings on medical implants	MARTINEZ PEREZ, David

12:00	DEVELOPMENT OF MULTIFUNCTIONAL HYALURONIC ACID HYDROGELS WITH ANTIBACTERIAL, ANTI-INFLAMMATORY AND NUCLEIC ACID DELIVERY PROPERTIES	GRIBOVA, Varvara
12:10	Effect of gallium doped hydroxyapatite on P. aeruginosa bacteria growth	MOSINA, Marika
12:20	3D PRINTED SCAFFOLDS WITH NON-ANTIBIOTIC ANTIMICROBIAL-DOPED HYDROXYAPATITE FOR INHIBITING S. AUREUS GROWTH IN VITRO AND SUPPORTING BONE REGENERATION IN VIVO	GENOUD, Katelyn

S13-1 Biofunctionalized surfaces for cellular and tissue engineering - Room: S2 (11:00 - 12:30)

-Conveners: Rui L. Reis

time	title	presenter
11:00	BIOMIMETIC SURFACE COATINGS AND HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS	GROTH, Thomas
11:20	Surface Functionalised Biomaterials and Nanostructures for Advanced Therapies	NEVES, Nuno
11:40	INTRODUCING CONTINUOUS MATERIAL GRADIENTS IN OSTEOCHONDRAL CONSTRUCTS VIA A NOVEL EXTRUSION-BASED 3D PRINT HEAD	BEEREN, Ivo
11:50	High-content image-based profiling for evaluating the effect of peptide coating effect on medical devices	SUGIYAMA, Ayato
12:00	bFGF-functionalized polyisocyanopeptide hydrogel for tissue regeneration of the pelvic floor	VAN VELTHOVEN, M.J.J.
12:10	Developing brain-targeting liposomes to deliver mesenchymal stem cells secretome for Parkinson's Disease Regenerative Medicine	BARATA-ANTUNES, Sandra
12:20	Guided cartilage formation: covalent growth factor immobilization on melt electrowritten microfiber scaffolds	AINSWORTH, Madison J.

S02 3D in vitro tissue-engineered cancer/disease models – Session II - Room: S1 (13:30 - 15:00)

-Conveners: Serena Danti; Rui L. Reis

time	title	presenter
13:30	Mimicking the tumor stroma-induced vasculature collapse in 3D pancreatic tumor model	PRAKASH, Jai
13:50	BOTTOMS-UP BIO-PRINTING OF CELLULARIZED POROUS MICRO-SCAFFOLDS TO ENHANCE CELL PROLIFERATION, VIABILITY AND MIGRATION	ROUSSELLE, Adrien
14:00	Post-printing structure formation in bioprinted tissue constructs that mimic the tumor microenvironment	NEAGU, Adrian
14:10	Bioreactor dynamic organotypic culture of primary liver cancer as a personalised immunocompetent drug screening platform for immuno-oncology	URBANI, Luca
14:20	Development of a bioprinted breast cancer model using decellularized mammary glands	BLANCO-FERNANDEZ, Barbara
14:30	INVESTIGATION OF BREAST CANCER EPITHELIAL-MESENCHYMAL TRANSITION USING 3D COLLAGEN-BASED MODELS	SAINSBURY, Elizabeth
14:40	ENGINEERING BIOMIMETIC HUMAN LUNG TUMOR MODELS	OZTURK, Ece
14:50	THE BIOMECHANICAL SIGNATURES OF 3D IN VITRO TUMOUR MODELS	MICALET, Auxtine

S09 Biobanking - indispensable support for the development of regenerative medicine - Room: S4 B (13:30 - 15:00)

-Conveners: Anna Chróścicka; Maria Chatzinikolaidou; Gilles Van Tienderen

time	title	presenter
13:30	Can biofabrication technologies help to facilitate biobanking of tissue engineered products?	MORONI, Lorenzo
13:50	TISSUE ENGINEERING AND BIOBANKING - A POSSIBLE FORCE-JOINING ALLIANCE IN APPLIED SCIENCES	LEWANDOWSKA-SZUMIEL, Malgorzata
14:10	Are there any differences between biobanking and banking of tissues and cells for clinical use?	KAMIŃSKI, Artur
14:20	Bone-forming capacity and immunogenicity of engineered and decellularized human cartilage grafts	PRITHIVIRAJ, Sujeethkumar
14:30	LIPID-POLYMER NANOCARRIERS FOR CARTILAGE REGENERATION	WYTRWAL-SARNA, Magdalena
14:40	The effect of Auxetic metamaterial scaffolds in osteogenic differentiation of Mesenchymal Stem Cells	FLAMOURAKIS, George
14:50	CRYOPRESERVED ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION FOR THE GROWTH FACTOR-FREE VASCULARIZATION OF BLUE SHARK COLLAGEN SPONGES	FREITAS RIBEIRO, Sara

S19 Biomimetic Approaches to Cardiovascular Regeneration: how and why? - Room: S4 A (13:30 - 15:00)

-Conveners: Petra Mela; Elena De-Juan-Pardo; Julia Marzi

time	title	presenter
13:30	Biomimetic approaches to heart valve engineering: ready to tell you how and at work to tell why.	D'AMORE, Antonio
13:50	MELT ELECTROWRITING FOR TUNING THE PROPERTIES OF IMPLANT SURFACES	BURKHARDT, Sarah
14:00	MELT-ELECTROWRITTEN HIGHLY TUNABLE ANISOTROPIC SCAFFOLDS FOR CARDIOVASCULAR TISSUE ENGINEERING	MUELLER, Kilian
14:10	Layered vascular grafts - mechanical properties and hemocompatibility	ŁOPIANIAK, Iwona
14:20	MESO-SCALE PATTERNED COLLECTING TARGET TO INDUCE LOCAL ANISOTROPY AND CURVILINEAR FIBER ORIENTATION IN ELECTRO-DEPOSITED, MICRO-FIBER BASED MITRAL VALVE SCAFFOLDS	TERRANOVA, Pietro
14:30	3D PRINTING AND MULTILAYERED ELECTROSPINNING - A NOVEL METHOD TO PRODUCE BIOMIMICKING HEART VALVES	BISCHOF, Lara
14:40	DEVELOPMENT OF A BIO-INSPIRED SCAFFOLD FOR SMALL Ø VASCULAR REGENERATION	FEDERICI, Angelica S.
14:50	Development of an advanced tissue-engineering system through novel 3D printing fabrication methods	IGLESIAS-GARCÍA, Olalla

S17 Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility - Room: S3 B (13:30 - 15:00)

-Conveners: Aleksandra Klimczak; Pierre Tournier

time	title	presenter
13:30	From Geometrical Patterns to Bioinspired Topographies: Nanofibrillar Microbundles Induce Strong Topological Modulation of Primary Human Immune Cells	GROLL, Jürgen
13:50	Cell Membrane Camouflage Mesoporous Bioactive Glass Nanoparticles embedding Glucose Oxidase for Targeted Enhanced Tumor Therapy	SUI, Baiyan

time	title	presenter
14:00	COMBINING BIOPRINTING AND MELT-ELECTROWRITING TECHNIQUES IN A MULTI-MATERIAL APPROACH FOR THE REPLACEMENT OF THE TEMPOROMANDIBULAR JOINT	CAIADO DECARLI, Monize
14:10	PREDICTION OF IN VITRO SCAFFOLD LIFETIME THROUGH THERMALLY-ACCELERATED AGEING AND FTIR SPECTROSCOPY	ROHMAN, Geraldine
14:20	VORONOI DESIGN OF ADDITIVELY MANUFACTURED 3D-PRINTED PCL-HA SCAFFOLDS: COMPREHENSIVE IN VITRO AND IN VIVO CHARACTERIZATION	LAUBACH, Markus
14:30	Harnessing the immunomodulation potential of nanoclay – an analysis of macrophage response	KIM, Yang-hee
14:40	Effects of subtoxic concentrations of various metal ions on mesenchymal stem/stromal cells	HAHN, Olga
14:50	INDUCED MESENCHYMAL STEM CELLS AS A SECRETOME SOURCE FOR CNS REGENERATIVE THERAPIES: SIMILAR SECRETORY PROFILE BUT DECREASED REPLICATIVE SENESCENCE COMPARED TO BONE MARROW MESENCHYMAL STEM CELLS	SANTOS, Diogo J.

S16-1 Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential - Room: S2 (13:30 - 15:00)

-Conveners: Sylvia Nürnberger; Andrea Barbero

time	title	presenter
13:30	Extracellular Matrix Derived Scaffolds for Cartilage and Osteochondral Defect Repair	KELLY, Daniel
13:50	CHARACTERIZING IN VIVO DEFORMATION DYNAMICS IN ORGAN SCAFFOLDS USING INTRAVITAL MICROSCOPY	CORRIDON, Peter
14:00	Development and characterisation of a novel 3D bioprinted biomimetic collagen and hyaluronic acid scaffold for the repair of cartilage defects	O'SHEA, Donagh
14:10	Decellularised pleural membranes in pulmonary regenerative medicine	VIKRANTH, Trisha
14:20	Designing a Peptide Hydrogel for Early Detection of Cancer	MAHON, Niall
14:30	Collagen/Pristine Graphene as an Electroconductive Interface Material for Neuronal Medical Device Applications	MAUGHAN, Jack
14:40	NOVEL HYPOXIA MIMICKING PEG-BASED NANO-BIOINK FOR CARTILAGE REGENERATION APPLICATION	RAVI, Subhashini
14:50	A scaffold-free graft for large critical size bone defect: preclinical evidence to clinical proof of concept	THEYS, Nicolas

S06 Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective - Room: S3 A (13:30 - 15:00)

-Conveners: Riccardo Levato; Carlos Mota

time	title	presenter
13:30	Dynamic hydrogels for biofabrication	BAKER, Matthew
13:50	BIOPRINTING ON-CHIP MICROPHYSIOLOGICAL MODELS OF HUMANIZED KIDNEY TUBULOINTERSTITIUM (BIRDIE)	MOTA, Carlos
14:00	Optically-tuned bioresins for the ultra-fast volumetric bioprinting of hepatic organoid-laden biofactories	NUNEZ BERNAL, Paulina

14:10	DEVELOPMENT OF CONDUCTIVE STIMULI-RESPONSIVE FIBROUS HYDROGELS FOR NEURAL INTERFACES	ZARGARIAN, Seyed Shahrooz
14:20	3D BIOPRINTED CONSTRUCTS TO GENERATE MATURE ORGANOIDS FROM IPSC-DERIVED RENAL PROGENITORS	ADDARIO, Gabriele
14:30	A biofabrication technology for generating multiscale channels in hydrogels for complex 3D in vitro co-cultures	SEIJAS-GAMARDO, Adrián
14:40	Multimaterial complex tissue models via suspension media-enhanced volumetric bioprinting	RIBEZZI, Davide
14:50	KIDNEY-ON-A-CHIP - INTEGRATING GLOMERULAR FILTRATION AND TUBULAR REABSORPTION MODELS	JÄSCHKE, Michelle

S25+S64 Cellular senescence in tissue damage and regeneration + Understanding and preventing early inflammatory events that lead to development of osteoarthritis - Room: S4 C (13:30 - 15:00)

-Conveners: Mikolaj Ogrodnik; Markus Schosserer; Melanie Hart

time	title	presenter
13:30	Cellular senescence during aging and chronic diseases: mechanisms and therapeutic opportunities	JURK, Diana
13:50	How to leverage cellular senescence for regeneration: a story of three salamanders	YUN, Maximina
14:10	Characterization of cellular senescence in development, ageing and wounding of mouse skin by creation and exploration of the largest sc-RNA-seq database of murine skin cells	ROZMARIC, Tomaz
14:20	CELLULAR SENESCENCE IMPAIRS CHONDROGENIC DIFFERENTIATION OF MSCS VIA TGFB SIGNALING INTERFERENCE	NARCISI, Roberto
14:30	A QUANTITATIVE TACK ON THE NANO CONSTRUCT FOR THE MODULATION OF INFLAMMATORY CYTOKINES IN BURN SCARS	PANNEERSELVAM MANIMEGALAI, Nivethitha
14:40	A COMPARTMENTALIZED JOINT-ON-CHIP MODEL TO UNRAVEL THE ROLE OF CARTILAGE AND SYNOVIUM IN OSTEARTHRITIS PATHOGENESIS	PALMA, Cecilia
14:50	Combination of IL-1β and IL-17A synergistically induce an early inflammatory and degenerative expression profile in healthy chondrocytes and synovial fibroblasts	HART, Melanie

S07-2 Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration - Room: S3 A (15:30 - 17:00)

-Conveners: Valeria Chiono; Michael Monaghan

time	title	presenter
15:30	Effectiveness of human iPSC-derived cardiomyocytes, but not stromal cells ("MSC"), for heart repair	DULAK, Józef
15:50	Injectable hydrogel for microRNA release in cardiac regenerative medicine	NICOLETTI, Letizia
16:00	BIOFABRICATION OF SCAFFOLD-FREE 3D CELLULAR STRUCTURES USING MAGNETIC LEVITATIONAL ASSEMBLY TO STUDY CARDIAC TOXICITY	ONBAS, Rabia
16:10	AN INDUCED PLURIPOTENT STEM CELL-BASED MODEL TO STUDY THE MECHANOBIOLOGY OF MYOCARDIAL FIBROSIS	NIRO, Francesco
16:20	ELECTROCONDUCTIVE SCAFFOLDS FOR IN VITRO CARDIAC MODELS	SOLAZZO, Matteo

16:30	Harnessing the Potential of Immune Cells to Promote Cardiac Repair Following Myocardial Infarction	ALSHOUBAKI, Yasmin
16:40	Design and fabrication of advanced thick human cardiac engineered tissues	MAZO-VEGA, Manuel M.
16:50	BIOMECHANICALLY STIMULATED 3D ENDOTHELIAL GUT-ON-CHIP PLATFORM TO STUDY INTESTINE MICROBIOME AND IMMUNE SYSTEM INTERACTIONS	KUGIEJKO, Karol

S16-2 Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential - Room: S2 (15:30 - 17:00)

-Conveners: Andrea Barbero; Sylvia Nürnberger

time	title	presenter
15:30	Whey Protein isolate: a multifunctional dairy-derived biomaterial	DOUGLAS, Timothy
15:50	HYDROLYTIC DEGRADATION CHARACTERIZATION OF 3D PRINTED POLYESTER SCAFFOLDS UNDER STATIC CONDITIONS AND FLOW PERFUSION	ALAMÁN-DÍEZ, Pilar
16:00	FIBRIN-BASED HYDROGELS WITH TUNEABLE MECHANICAL PROPERTIES	AL ENEZY-ULBRICH, Miriam Aischa
16:10	IMPROVED CELLULAR INFILTRATION BY GLYCOSAMINOGLYCANS REMOVAL AND ALTERED STIFFNESS - A STUDY ON AURICULAR CARTILAGE SCAFFOLDS.	CASADO LOSADA, Isabel
16:20	THE PREPARATION AND CHARACTERISATION OF POLY(3-HYDROXYBUTYRATE-co-4-HYDROXYBUTYRATE) [P(3HB-co-4HB)] BASED BIOCOMPOSITE FOR TRANSLATIONAL BIOMEDICAL APPLICATIONS	ALIAA, Nik
16:30	HUMAN EPIDERMAL SKIN EQUIVALENTS	BOYADJIEV, Alexander
16:40	PRODUCTION OF HIGHLY ANGIOGENIC HYDROGELS FROM THE EXTRACELLULAR MATRIX OF CULTURED STROMAL VASCULAR FRACTION OF ADIPOSE TISSUE	VILAÇA-FARIA, Helena
16:50	HOW NATURAL BIOMATERIAL CONSISTENCY LEADS TO PREDICTABILITY AND TUNABILITY	ZEGWAART, Jan-Philip

S15-1 Biologically inspired and Engineered disease models - Room: S1 (15:30 - 17:00)

-Conveners: Andrew Daly

time	title	presenter
15:30	Humanized platforms by convergence of biomaterials, cells and microtechnologies	YESIL-CELIKTAS, Ozlem
15:50	Tuning macrophage polarization to model myocardial infarction in the generation of functional cardiac organoids	SUKU, Meenakshi
16:00	RECONSTRUCTION OF FUNCTIONAL GRADIENTS USING MELT ELECTROWRITING	WŁODARCZYK-BIEGUN, Małgorzata
16:10	Tissue engineering a humanized rat model for osteosarcoma research	HUTMACHER, Dietmar W.
16:20	ELECTROSPUN PATCH DELIVERY OF ANTI-TNFα F(ab) ANTIBODY FRAGMENT FOR THE TREATMENT OF ORAL MUCOSAL INFLAMMATORY DISEASES	EDMANS, Jake
16:30	DOX-LOADED MPEG NANOPARTICLES AS A PROMISING TREATMENT IN A HUMANIZED MOUSE MODEL FOR BREAST CANCER BONE METASTASIS	FRANKENBACH, Tina

16:40	A 3D IN VITRO MODELS OF IMPAIRED OSTEOCYTES ACTIVITY UNDER EXPSOURE TO INDOXYL SULFATE	MIHĂILĂ, Silvia Maria
16:50	GLYCOTRIPEPTIDES SHOWCASE THE EFFECT OF GLYCOSYLATION ON PROTEIN AGGREGATION	BRITO, Alexandra

S22 Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine - Room: S4 C (15:30 - 17:00)

-Conveners: Yuto Takemoto; Bernd Rolauffs

time	title	presenter
15:30	Image-based label-free analysis for quantitative and real-time understanding of cellular status	KATO, Ryuji
15:50	Basics of Cellular and Subcellular Mechanobiology	SCHLUNCK, Günther
16:10	CHONDROCYTE PROLIFERATION IS INFLUENCED MORE BY F-ACTIN DENSITY AND THE MACROSCOPIC TISSUE DISEASE STATE THAN BY CELL SHAPE OR MICROPATTERN GEOMETRY	ROLAUFFS, Bernd
16:20	Morphology-based detection of senescence in expanded mesenchymal stem cells	TAKEMOTO, Yuto
16:30	Using a machine learning-supported approach for assessing and predicting the susceptibility of articular cartilage to mechanical trauma-induced changes in cellularity	SELIG, Mischa
16:40	PREDICTION OF M1, M2A AND M2C MACROPHAGE PHENOTYPES AND THEIR IL-10 PRODUCTION POTENTIAL BASED ON SINGLE CELL MORPHOLOGY AND PROTEIN INTENSITY USING A NOVEL MACHINE-LEARNING BASED APPROACH	POEHLMAN, Logan
16:50	PREDICTION OF MEDICAL DEVICE COATING PROPERTIES VIA MACHINE LEARNING	GRIBOVA, Varvara

S04 3D Writing Within Suspension Media for Tissue Engineering and In Vitro Modeling - Room: S4 B (15:30 - 17:00)

-Conveners: Rui M. A. Domingues; Manuela E. Gomes

time	title	presenter
15:30	Bioprinting high cell-density tissue models through spheroid fusion in self-healing hydrogels	DALY, Andrew
15:50	3D printed anisotropic and porous dense collagen hydrogels to model skeletal muscle extracellular matrix	CAMMAN, Marie
16:00	CHEMICALLY FUNCTIONALIZABLE AND MECHANICALLY TUNABLE BIOMATERIAL FOR EMBEDDED 3D BIOPRINING	BECKER, Malin Lea
16:10	An open source extrusion bioprinter based on the E3D motion system and tool changer to enable FRESH and multimaterial bioprinting	STELZL, Christina
16:20	High resolution light-based 3D printing of cell-laden bio constructs	MADRID-WOLFF, Jorge
16:30	Magnetically-Assisted 3D Bioprinting of Tissue Engineered Tendons	PARDO MONTERO, Alberto
16:40	Development of bioprinted osteochondral tissue: an in-vitro model for drug discovery	JAHANGIR, Shahrbanoo

S03+S33 3D printing of bionic organs – how far are we from clinical application? + From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine - Room: S3 B (15:30 - 17:00)

-Conveners: Marta Klak; Jakub Rybka; Lukasz Witek; James E. Smay; Anahita Ahmadi Soufivand

time	title	presenter
15:30	3D-bioprinted bionic pancreas as an innovative method of treating and preventing diabetes – how far we are from clinical aplication?	WSZOŁA, Michał
15:50	Tissue Engineered Scaffolds For Tracheal Regeneration: A seeding approach in a multi-layered 3D printed scaffold	SORIANO, Luis
16:00	Bone Regenerative Capacity of 3D Printed Bioactive Ceramic Scaffolds Coated with Bioactive Molecule: Dipyridamole	WITEK, Lukasz
16:10	SCAFFOLD GUIDED BONE TISSUE ENGINEERING FOR THE ASSESSMENT OF BONE DEFECT RECONSTRUCTION – PRE-CLINICAL AND CLINICAL TRIALS	MEDEIROS SAVI, Flavia
16:20	SELF-ASSEMBLING PEPTIDE HYDROGELS AS BIOINKS FOR 3D BIOPRINTING APPLICATIONS	GINJAUME, Albert
16:30	BIODEGRADABLE AND BIOACTIVE PERSONALIZED IMPLANT FOR GUIDED BONE REGENERATION	REY-VIÑOLAS, Sergi
16:40	BONE REGENERATION EXPLOITING CORTICOPERIOSTEAL TISSUE TRANSFER FOR SCAFFOLD-GUIDED BONE REGENERATION	HUTMACHER, Dietmar W.
16:50	Meniscus regeneration of the future. From the slaughterhouse, through cell culture to 3D bioprinting.	RYBKA, Jakub

S68 Human brain organoids versus assembloids approach for neurodevelopmental studies - Room: S4 A (15:30 - 17:00)

-Conveners: Arti Ahluwalia; Leonora Bużańska; Chiara Rinoldi

time	title	presenter
15:30	Development of the integrated human brain organoids	PARK, In-Hyun
15:50	In vitro modeling of human brain region interactions	REUMANN, Daniel
16:10	ADVANCED IN SILICO METHODS FOR ORGANOID AND ASSEMBLOID DESIGN	MAGLIARO, Chiara
16:20	PHYSIOLOGICAL NORMOXIA INFLUENCE NEURAL CELL FATE THROUGH CHANGES OF MITOCHONDRIAL DYNAMICS AND GLYCOLYSIS/OXPHOS SWITCH IN HUMAN BRAIN ORGANOID MODEL	LIPUT, Michal
16:30	Establishing tools to study the emergence of cellular diversity in the human brain	NOWAKOWSKI, Tomasz
16:50	Round Table Discussion (10 minutes)	

Awards Session - Room: S1 (17:30 - 19:00)

General Assembly - Room: S1 (19:00 - 20:00)

Wednesday, 29 June 2022

P1 Plenary Session: Gerjo van Osch (plenary lecture) Cartilage regeneration: the challenges of regenerating a “simple” non-vascularised tissue - Room: S1 (09:00 - 10:00)

-Conveners: Geoff Richards

time	title	presenter
09:00	Cartilage regeneration: the challenges of regenerating a “simple” non-vascularised tissue	VAN OSCH, Gerjo

Debate 1: Regeneration of human joints (Prof. Alicia El Haj, Prof. Fergal O'Brien, Prof. Geoff Richards, Prof. Gerjo van Osch) - Room: S1 (10:00 - 10:30)

-Conveners: Martin Stoddart

time	title	presenter
10:00	Debate: Regeneration of human joints	EL HAJ, Alicia O'BRIEN, Fergal RICHARDS, Geoff VAN OSCH, Gerjo

S10-1 Biofabricated Tissues and Organs for Clinical Impact - Room: S1 (11:00 - 12:30)

-Conveners: Andrew Daly; Laura De Laporte

time	title	presenter
11:00	Biofabricated Articular and Cardiac Tissues for Clinical Impact	MALDA, Jos
11:20	PHYSIOMIMETIC CULTURE OF MESENCHYMAL STROMAL CELLS AFFECTS MACROPHAGE ACTIVITY IN A PARACRINE MANNER	FALCONES, Bryan
11:30	Engineered and decellularized human cartilage grafts instruct full regeneration of critical-sized femoral defects	GARCIA GARCIA, Alejandro
11:40	A WOVEN VASCULAR GRAFT PRODUCED FROM YARN OF HUMAN AMNIOTIC MEMBRANE	L'HEUREUX, Nicolas
11:50	Philosophy of science, a tool to face engineered liver challenges	GUILLET, Manon
12:00	TOWARDS FABRICATION OF A TRIPLE CULTURE LIVER SINUSOID MODEL UTILIZING 3D CORE-SHELL BIOPRINTING	LODE, Anja
12:10	LIVER MATRIX AND PERFUSION BIOREACTOR CULTURE PROMOTE AMNION EPITHELIAL CELL DIFFERENTIATION INTO FUNCTIONAL HEPATOCYTES	CAMPINOTI, Sara
12:20	A modular bioreactor for dynamic culturing of human multilayer tissues structures	GASPERINI, Luca

S12 Biofabrication with light-based technologies and high-definition printing - Room: S3 B (11:00 - 12:30)

-Conveners: Tiziano Serra; Marcy Zenobi-Wong

time	title	presenter
11:00	Light-driven technologies to steer the functionality of volumetric engineered tissues and organoids	LEVATO, Riccardo
11:20	LASER-BASED HIGH-RESOLUTION 3D PRINTING AND BIOPRINTING FOR TISSUE ENGINEERING	OVSIANIKOV, Aleksandr

11:40	HARNESSING MICROFLUIDIC BIOPRINTING TO FABRICATE GRADIENT-LIKE POROUS 3D CONSTRUCTS VIA EMULSION INK DEPOSITION	MARCOTULLI, Martina
11:50	BOTTOM-UP TISSUE ENGINEERING BASED ON MICROSCAFFOLDS PRODUCED BY HIGH-RESOLUTION 3D PRINTING	KOPINSKI-GRÜNWALD, Oliver
12:00	DEFINED-GEOMETRY MICROPARTICLES PRODUCED BY TWO-PHOTON POLYMERISATION FOR SKELETAL APPLICATIONS	OWEN, Robert
12:10	Microfluidics-assisted bioprinting of double-emulsion droplets	TERRAZAS MALLEA, Ronald
12:20	EFFECT OF LIGHT STIMULI IN VOLUMETRIC BIOPRINTING ON CELL FUNCTIONALITY AT SINGLE CELL LEVEL	GUEYE, Marième

S48 Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy - Room: S4 A (11:00 - 12:30)

-Conveners: Joanna Idaszek; Akon Higuchi

time	title	presenter
11:00	MICROPATTERNED SURFACES FOR CONTROLLING STEM CELLS MORPHOLOGY AND FUNCTIONS	CHEN, Guoping
11:20	HYALURONIC ACID BASED NEXT-GENERATION BIOINK FOR 3D BIOPRINTING OF A HUMAN STEM CELL DERIVED CORNEAL STROMA EQUIVALENT AND A 3D CORNEA TISSUE MODEL WITH INNERVATION	MÖRÖ, Anni
11:30	DEVELOPMENT OF AN IPSC LOADED BIOMIMETIC SCAFFOLD SYSTEM FOR SPINAL CORD APPLICATIONS	O' CONNOR, Cian
11:40	ROAD TO UNIVERSAL ORGANS: DECELLULARIZED LIVER REPOPULATION WITH HLA I-II KNOCKOUT HEPATOCYTES IN A DYNAMIC BIOREACTOR CULTURE	CACIOLLI, Lorenzo
11:50	MULTIFUNCTIONAL 3D BIOPRINTING FOR TISSUE INTERFACES	ŞENTÜRK, Efsun
12:00	LUNG TISSUE TYPE SELECTED AMNIOTIC FLUID DERIVED MESENCHYMAL STEM CELLS FOR TREATMENT OF BLEOMYCIN INDUCED PULMONARY FIBROSIS IN A RAT MODEL	TALTS, Jan
12:10	Interplay between adipose-derived stem cells and inflammatory mediators: impact on neurite outgrowth and vascular morphogenesis	L. AFONSO, João
12:20	TOWARDS APPLICATION OF CELL THERAPY USING hiPSC-DERIVED MSCs AS A STABLE 'OFF-THE-SHELF' CELL SOURCE	RAMOS, Yolande F. M.

S28 Emerging and future technologies for peripheral nerve regeneration - Room: S4 C (11:00 - 12:30)

-Conveners: Srinivas Madduri; Neha Tiwari

time	title	presenter
11:00	Unveiling the Multiple Roles of Stem Cells Secretome in Nerve Regeneration	SALGADO, Antonio
11:20	Novel bioengineering approach for enhancing the nerve tissue regeneration process	MADDURI, Srinivas
11:40	THREE-DIMENSIONAL SCAFFOLDS BY MULTI-PHOTON POLYMERIZATION AS A CO-CULTURE SYSTEM FOR TISSUE REGENERATION	KORDAS, Antonis
11:50	An advanced nerve guidance conduit for repairing large peripheral nerve defects	KOCI, Zuzana

time	title	presenter
12:00	ALIGNED AND CONDUCTIVE 3D COLLAGEN/PPY SCAFFOLDS FOR PERIPHERAL NERVE TISSUE ENGINEERING	TRUEMAN, Ryan
12:10	EXTRACELLULAR VESICLES IN PERIPHERAL NERVE REGENERATION: EXTRACELLULAR VESICLES DERIVED FROM ADIPOSE STEM CELLS INCREASE SCHWANN CELL PROLIFERATION FOLLOWING INTERNALIZATION	HAERTINGER, Maximilian

S62 Tissue regeneration by integration of bioinspired materials - Room: S4 B (11:00 - 12:30)

-Conveners: Sandra Van Vlierberghe; Heungsoo Shin

time	title	presenter
11:00	TBA	RODRÍGUEZ-CABELLO, José Carlos
11:20	The Controlled Delivery of Proteoglycan-4 in a Scaffold-Based System for Cartilage Repair Applications	MATHESON, Austyn
11:30	Hybrid 3D-printed hydrogel scaffolds for liver tissue engineering	CARPENTIER, Nathan
11:40	Combining proteolytic sequences, VEGF-mimetic peptide and laminin-derived peptide within Elastin-Like Recombinamer scaffolds for the spatiotemporal direction of angiogenesis and neurogenesis	GONZÁLEZ-PÉREZ, Fernando
11:50	TIME COURSE OF ECTOPIC BONE FORMATION IN RATS INDUCED BY rhBMP6 WITHIN AUTOLOGOUS BLOOD COAGULUM WITH CALCIUM PHOSPHATE CERAMIC PARTICLES	STOKOVIC, Nikola
12:00	Prognostic evaluation of the use of three-dimensional (3D) scaffolds on chronic skin lesions using new biomedical imaging technologies.	CAVALLINI, Chiara
12:10	HEPARAN SULPHATE ANALOGUE HYDROGELS AS A PLATFORM FOR KIDNEY ORGANOID MATURATION	MOTA, Carlos
12:20	DIRECTING STEM CELL COMMITMENT IN 3D BIOINSPIRED HYDROGELS BY GROWTH FACTOR SEQUESTRATION USING MOLECULARLY IMPRINTED NANOPARTICLES	TEIXEIRA, Simão P. B.

S43-1 Multifunctional biomaterials supporting bone regeneration - Room: S2 (11:00 - 12:30)

-Conveners: Timothy Douglas; Elżbieta Pamuła

time	title	presenter
11:00	Current status and future prospects of genome-scale metabolic modeling to optimize the use of mesenchymal stem cells in regenerative medicine	SIGURJÓNSSON, Olafur
11:20	OSTEOINDUCTIVE INJECTABLE CALCIUM PHOSPHATE BIOACTIVATED BY PHOSPHOSERINE DENDRONS	GRAZIA RAUCCI, Maria
11:40	MECHANICAL STIMULATION PROMOTES THE OSTEOGENIC RESPONSE OF PRE-OSTEOBLASTS ON POLYMERIC SCAFFOLDS	CHATZINIKOLAIDOU, Maria
11:50	Biofabrication of the vascularised osteogenic niche	PARMENTIER, Laurens
12:00	Calcium phosphate based biomaterials influence on cell metabolism	FAN, Jingzhi
12:10	Evaluation of β tricalcium phosphate and poly(3-hydroxybutyrate) -based scaffolds for bone tissue regeneration	SKIBIŃSKI, Szymon
12:20	OSTEOGENIC ACTIVITY OF ADDITIVE MANUFACTURED TITANIUM ALLOY-CALCIUM PHOSPHATE CERAMIC SCAFFOLDS FOR CRANIOPLASTY IN VITRO AND IN A LARGE ANIMAL CALVARIAL DEFECT MODEL	KOPER, David

S13-2 Biofunctionalized surfaces for cellular and tissue engineering - Room: S3 A (11:00 - 12:30)

-Conveners: Rui L. Reis

time	title	presenter
11:00	ELECTROACTIVE POLYCAPROLACTONE-GRAPHENE NANOCOMPOSITES COMBINED WITH ZINC IONS TRIGGER MYOGENIC DIFFERENTIATION	APARICIO COLLADO, Jose Luis
11:10	Probing T Cell Mechanosensitivity using Artificial Antigen-Presenting Cells	ALATOOM, Aseel
11:20	Cell-selective adhesion short peptides for enhancing cell culture on scaffold	FUJIMOTO, Akiyo
11:30	ANTIBACTERIAL ALBUMIN-TANNIC ACID COATINGS FOR SCAFFOLD-GUIDED BREAST RECONSTRUCTION	COMETTA, Silvia
11:40	POLY(ARGININE) AND HYALURONIC ACID FILM: A MULTIFUNCTIONAL COATING FOR SCAFFOLDS AND INVASIVE MEDICAL DEVICES: THE CASE OF CAVI-T INTRANASAL BALLOON	CALLIGARO, Cynthia
11:50	Innovative Hydrogel to Overcome the Glioblastoma Therapy Deadlock	SUSANA COSTA MACHADO FERREIRA, Helena
12:00	BUILDING BARRIERS: ENGINEERING A NOVEL IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER	SCHOFIELD, Christina
12:10	Novel Elastomer Surface Modification Technique for Corneal Limbal Epithelial Stem Cell Investigation	DIMMOCK, Ryan

S30 European regional platforms for TERM - Update - Room: S4 C (13:30 - 15:00)

-Conveners: Gerjo van Osch; Heinz Redl

time	title	presenter
13:30	Belgium Example - Gent Platform Advanced Therapies and Tissue Engineering	AMONS, Gudrun
13:40	UK Example - Regenerative Medicine Platform UKRMP II	OREFFO, Richard
13:50	REGENERATIVE MEDICINE AND TECHNOLOGY – A NEW BACHELOR PROGRAM	BAUER, Jurica
14:00	Netherland Example - RegMedXB	MULDER, Bernard
14:10	Ireland Example - CURAM-A National Center for Research in Medical Devices	PANDIT, Abhay
14:20	Austrian Example - Austrian Cluster for Tissue Regeneration	REDL, Heinz
14:30	Round table discussion (30 minutes)	

S43-2 Multifunctional biomaterials supporting bone regeneration - Room: S2 (13:30 - 15:00)

-Conveners: Elżbieta Pamuła; Timothy Douglas

time	title	presenter
13:30	CONTROLLED DELIVERY OF EPIGENETICALLY ACTIVATED EXTRACELLULAR VESICLES FROM A GELMA/NANOCLAY HYDROGEL FOR BONE REGENERATION	MAN, Kenny
13:40	BONE REGENERATION OF A CRITICAL-SIZED DEFECT IN SHEEP WITH A 3D PRINTED SCAFFOLD COATED WITH A BIOMETIC FILM CONTAINING LOW-DOSE OF BMP-2	SCHOFFIT, Sarah
13:50	New surface functionalities from grafting natural biomolecules to titanium alloys	GAMNA, Francesca
14:00	ENGINEERING OF A BRIDGE PROTEIN TO IMPROVE THE DELIVERY OF BMP-2 FROM COLLAGEN SPONGE AND ENHANCE BONE REGENERATION FOR SPINAL FUSION	BRIQUEZ, Priscilla

14:10	PCL reinforced collagen scaffolds for endochondral healing of bone defects	LEEMHUIS, Hans
14:20	MICROSTRUCTURE EFFECT ON BONE FORMATION OF A FUNCTIONALLY GRADED SCAFFOLD USING A MECHANOSTAT-BASED MODEL	ALIPOUR GHASSABI, Ata
14:30	EFFECT OF 3D SCAFFOLD MORPHOLOGY ON BONE TISSUE REGENERATION BASED ON A MULTI-PHYSICS FEM MODEL	OZTURK, Sezen
14:40	DELIVERY OF MESENCHYMAL STROMAL CELLS USING COLLAGEN MEMBRANES EMBEDDED IN LEGO®-INSPIRED MULTICOMPONTENT SCAFFOLDS FOR PERSONALISED MANDIBULAR DEFECT REPAIR	PHELIPE HATT, Luan
14:50	Composite Biomaterial-Ink with Hyaluronan, Collagen and Calcium Phosphate Particles for Delivery of Chemically Modified RNA to promote Bone Regeneration	VAN DER HEIDE, Daphne

S24 Cell-rich constructs for tissue engineering - Room: S1 (13:30 - 15:00)

-Conveners: Christina Schofield; Manuel Salmeron-Sanchez; Elana Meijer

time	title	presenter
13:30	High cells/biomaterials ratio approaches in tissue engineering	MANO, João
13:50	PAPILLARY AND RETICULAR FIBROBLASTS GENERATE DISTINCT MICROENVIRONMENTS THAT DIFFERENTIALLY IMPACT ANGIOGENESIS	MULLER, Laurent
14:00	AN IN VITRO IMMUNOCOMPETENT HUMAN TISSUE-ENGINEERED MODEL OF ATOPIC DERMATITIS FOR DRUG TESTING	BARRAGAN VAZQUEZ, Inmaculada
14:10	IS MORE ALWAYS BETTER? MODULATING HUMAN ADIPOSE DERIVED STROMAL CELLS CHONDROGENESIS TO ACHIEVE OPTIMAL BONE REMODELING IN VIVO	CHAABAN, Mansoor
14:20	LAMINARAN/PLATELET LYSATE-BASED HYDROGELS: TOO GOOD TO BE TRUE	ZARGARZADEH, Mehrzad
14:30	Perfusion Flow on urogenital epithelial cells for urethral tissue engineering purposes	DE GRAAF, Petra
14:40	INTERLEUKIN 1 BETA MODULATES THE EQUINE TENOCYTE TRANSCRIPTOME IN 3D CULTURE BY ENHANCING NF-KB SIGNALLING	BEAUMONT, Ross
14:50	Optimisation of bioprocessing conditions for an implantable myoblast-microcarrier combination for treatment of incontinence	CARTAXO, Ana Luísa

S37 Human Organoids for Musculoskeletal Tissues - Room: S4 A (13:30 - 15:00)

-Conveners: Debby Gawlitta; Xiao-hua Qin

time	title	presenter
13:30	Engineering Grafts for Joint Regeneration using Phenotypically Distinct Cartilaginous Microtissues	KELLY, Daniel
13:50	Structural support for human cartilage organoids	MALDA, Jos
14:10	Microengineered 3D Bone Cell Models via Image-guided Two-photon Subtractive Lithography	QIN, Xiao-hua
14:20	Increased cell density increases mineral formation rates and stiffness in 3D bioprinted patient-derived bone organoids using dynamic loading	DE LEEUW, Anke
14:30	Directing human mesenchymal stem cells differentiation towards hypertrophic chondrocytes using fiber-reinforced bone dECM hydrogel scaffolds	IDASZEK, Joanna

time	title	presenter
14:40	THE INTERPLAY BETWEEN IMMUNE RESPONSE AND BONE FORMATION FROM DEVITALIZED ALLOGENEIC CELLS	DE SILVA, Leanne
14:50	TOWARDS BONE-REMODELING-ON-A-CHIP: FORMATION OF 3D BONE-LIKE TISSUES	VIS, Michelle

S31 Extracellular vesicles – next generation tool for diagnostics and regenerative medicine - Room: S3 A (13:30 - 15:00)

-Conveners: Ewa Zuba-Surma; Barbara Łukomska; Dario Manzanares Sandoval

time	title	presenter
13:30	UNSOLVED MYSTERIES AND CURRENT OPPORTUNITIES IN EXTRACELLULAR VESICLES	WITWER, Kenneth
13:50	MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES AND THEIR FUNCTIONAL HETEROGENEITY	GIEBEL, Bernd
14:10	EXTRACELLULAR BIOADDITIVES-ADJUVANTED INJECTABLE HYDROGEL SUPPORTS NEOANGIOGENESIS AND DAMPENS ADVERSE CARDIAC REMODELLING	MAIULLARI, Fabio
14:20	EXTRACELLULAR VESICLES FROM HUMAN IPS CELLS ENHANCE RECONSTITUTION CAPACITY OF CORD BLOOD-DERIVED HEMATOPOIETIC STEM AND PROGENITOR CELLS	KARNAS, Elżbieta
14:30	INTRA-TRACHEAL INJECTION OF HUMAN EXTRACELLULAR VESICLES BLOCKS FIBROSIS AND REGENERATES EPITHELIAL LUNG CELLS IN A RAT MODEL OF BRONCHOPULMONARY DYSPLASIA	MAGAROTTO, Fabio
14:40	DEVELOPMENT OF BIOINSPIRED PROTEOLIPOSOMES AND CELL-DERIVED NANOVESICLES AS OSTEOGENIC SYNTHETIC EXTRACELLULAR VESICLES FOR BONE REGENERATION	BRUNET, Mathieu Y.
14:50	SECRETOME OF ADIPOSE TISSUE DERIVED STEM CELLS AND ELECTRICAL EPIDURAL STIMULATION PROMOTES FUNCTIONAL GAINS IN SPINAL CORD INJURY CONTEXT	RIBEIRO, Jorge

S38 Injectable biomaterials for cell-instructive matrix cues - Room: S3 B (13:30 - 15:00)

-Conveners: Mirosława El Fray

time	title	presenter
13:30	ENGINEERING INJECTABLE THERAPEUTIC BIOMATERIALS FOR MUSCO-SKELETAL TISSUE REPAIR/REGENERATION	AMBROSIO, Luigi
13:50	In situ assembling biohybrid polymer hydrogels to modulate cell-instructive matrix cues	WERNER, Carsten
14:10	FIREFLY-INSPIRED BIOMATERIALS AS TUNABLE, TRIGGERABLE, AND CELL-INSTRUCTIVE MATRICES FOR 3D CELL ENCAPSULATION	PAEZ, Julieta
14:20	Engineering Cell-Instructive Microenvironments Using Injectable, Topographically-Textured Polymeric Matrices	AMER, Mahetab
14:30	DEVELOPMENT OF IN SITU CROSSLINKABLE BIORESPONSIVE ALGINATE HYDROGELS	V. MAGALHÃES, Mariana
14:40	Injectable nanofibrous microscaffolds for cell and drug delivery	NAKIELSKI, Paweł
14:50	Clickable amphiphile alginate produces dynamic 3D cell microenvironments with microstructured hydrophobic domains	NEVES, Mariana I.

S20 Biomimetic in vitro models for bone regeneration and cancer pathologies - Room: S4 B (13:30 - 15:00)

-Conveners: Silvia Farè; Gabriela Graziani

time	title	presenter
13:30	Engineering 3D Human Multicellular Bone Models as Anti-metastastic Drug Screening Platforms	MORETTI, Matteo
13:50	In vitro testing of bone biomaterials - opportunities and challenges	STODDART, Martin
14:10	IN VITRO BONE MARROW NICHE FOR METASTASIS ASSAY	WENTA, Tomasz
14:20	Bridging the gap between the immune response and mineralization during fracture healing	LACKINGTON, William
14:30	Biogenic and biomimetic nanocoatings for bone modelling and regeneration	GRAZIANI, Gabriela
14:40	Algorithmic Engineering enabling Organotypical Print Templates at Scale	ERBEN, Amelie
14:50	Biofabrication of tumor models that mimic the tumor microenvironment using extrusion bioprinting	ARJOCA, Stelian

S47 New insights underlying mesenchymal stem cell-mediated bone regeneration - Room: S2 (15:30 - 17:00)

-Conveners: Kamal Mustafa; Cecilie Gjerde

time	title	presenter
15:30	STEM CELLS IN BONE REGENERATION, A RANDOMIZED CLINICAL TRIAL	GJERDE, Cecilie
15:50	Bone-Marrow Mesenchymal Stem/Stromal Cells Have Enhanced Vasculogenic Potency Over Adipose Stem/Stromal Cells in Perfused In Vitro Cultures	MIETTINEN, Susanna
16:10	Extracellular Vesicles Secreted by Osteogenic-Differentiated Mesenchymal Stem Cells Promote Bone Formation In Rat Calvarial Defect	MUSTAFA, Kamal
16:20	DEVELOPMENT OF ANGIOGENIC BIOINK FOR VASCULARIZED BONE TISSUE ENGINEERING	KORKEAMÄKI, Jannika
16:30	MACROPHAGE MEDIATED IMMUNOMODULATION BY EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS COMBINED WITH BIPHASIC CALCIUM PHOSPHATE GRANULES FOR BONE REGENERATION	RANA, Neha
16:40	THE INFERIOR IN VIVO OSTEOGENICITY OF HUMAN MSC FROM ADIPOSE TISSUE COMPARED TO BONE MARROW IS CORRELATED WITH HIGHER IMMUNE RESPONSE WITHIN TISSUE ENGINEERED CONSTRUCTS	LOGEART-AVRAMOGLOU, Delphine
16:50	Fluid-flow mediated cytoskeletal adaptation regulates the growth and fate of bone marrow mesenchymal stem cells	YAMADA, Shuntaro

S41 Mesenchymal Stem / Stromal Cells - from basic research through clinical studies to registered products - Room: S3 A (15:30 - 17:00)

-Conveners: Marcin Majka; Ewa Zuba-Surma

time	title	presenter
15:30	MSC THERAPY: CLINICAL EVIDENCE AND SCIENTIFIC PROGRESS	DAWN, Buddhadeb
15:50	CONTROLLED DRUG RELEASE FOR TREATING SCI: TARGETING NEUROBIOLOGY MECHANISM IDENTIFIED BY STEM CELL-BASED MULTIMODAL APPROACHES	TENG, Tang D.
16:10	SURVIVING MESENCHYMAL STEM/STROMAL CELLS UPON INTRA-ARTICULAR DELIVERY IN AN OSTEOARTHRITIC JOINT EXPRESS A NEW CHONDROPROGENITOR GENE BMP/RETINOIC ACID-INDUCIBLE NEURAL-SPECIFIC PROTEIN 3 (BRINP3)	IVANOVSKA, Ana

time	title	presenter
16:20	EFFECT OF DIFFERENT LIGHT WAVELENGTHS ON ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/STROMAL CELLS	SRIDHARAN, Kaarthik
16:30	MULTIPLE WHARTON JELLY MESENCHYMAL STEM CELLS-DERIVED HE-ATMP TRANSPLANTATIONS OVERCOMES DRUG-RESISTANT EPILEPSY IN CHILDREN	MILCZAREK, Olga
16:40	CHAOTIC PRINTING OF HYDROGEL CARRIERS FOR MESENCHYMAL STEM CELL PROLIFERATION	DEAN, David
16:50	TAKING A STEP AHEAD: ENDOCHONDRAL BONE REGENERATION OF A CRITICAL SIZE DEFECT IN A LARGE ANIMAL MODEL	STAUBLI, Flurina

S11 Biofabrication using extrinsic fields - Room: S3 B (15:30 - 17:00)

-Conveners: Tiziano Serra; Luis Soriano

time	title	presenter
15:30	Ultrasound-based assembly of tissues and biomaterials	ARMSTRONG, James
15:50	HIGH-RESOLUTION TWO-PHOTON POLYMERIZATION OF ENGINEERED CELL MICROENVIRONMENTS FOR FUNDAMENTAL NEURO-MECHANOBIOLOGY AND BRAIN CANCER PROTON RADIOTHERAPY	ACCARDO, Angelo
16:10	4D BIOFABRICATION OF NERVE GUIDE CONDUITS USING RESPONSIVE MATERIALS	TIWARI, Neha
16:20	ENGINEERING DORSAL ROOT GANGLION MULTICELLULAR SYSTEM TOWARDS IN VIVO CROSS EXCITATION FUNCTION	MA, Junxuan
16:30	CONTROLLING THE SHAPE OF MICROCAPILLARY NETWORKS IN 3D IN VITRO MODELS THROUGH SOUND PATTERNING	DI MARZIO, Nicola
16:40	EFFECT OF SECOND STAGE HEATER ON MEW PROCESSING PARAMETERS	CHANDRAKAR, Amit
16:50	Cell density matters: Local cell density enhancement by sound to increase the therapeutic efficacy in regenerative medicine	GÉRALDINE GUEX, Anne

S27+S56 Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses + Skin wound healing in 2022: where basic science meets clinical needs - Room: S4 B (15:30 - 17:00)

-Conveners: Farzaneh Moghtader; Alexandra P. Marques

time	title	presenter
15:30	TBA	TÉOT, Luc
15:50	Multifunctional Bio-hybrids Composed of Gelatin Microspheres Carrying Bacteriophages and/or bFGF and Their Aggregates with Mesenchymal Stem Cells	MOGHTADER, Farzaneh
16:10	3D in vitro M2 macrophage model to mimic modulation of tissue repair	SAPUDOM, Jiranuwat
16:20	IN VITRO COMPARISON OF SELF-ASSEMBLED AND PLASMA-BASED TISSUE ENGINEERED SKIN SUBSTITUTES: TWO DIFFERENT MANUFACTURING PROCESSES FOR THE TREATMENT OF SEVERE BURN PATIENTS	SIERRA-SÁNCHEZ, Álvaro
16:30	Intradermal adipocytes differentiation and lipid metabolism are regulated through epidermal transcription factor Foxn1	WALENDZIK, Katarzyna
16:40	Dense Collagen/PLGA Composite Hydrogels Generated by In Situ Nanoprecipitation as Novel Medicated Wound Dressings: In Vitro and In Vivo Evaluation	HELARY, Christophe

time	title	presenter
16:50	HATMSC SECRETED FACTORS IN THE HYDROGEL AS A POTENTIAL TREATMENT FOR CHRONIC WOUNDS—IN VITRO STUDY	KRASKIEWICZ, Honorata

S15-2 Biologically inspired and Engineered disease models - Room: S1 (15:30 - 17:00)

-Conveners: Y. Shrike Zhang

time	title	presenter
15:30	INVESTIGATING THE EFFECT OF APOLIPOPROTEIN E4 ON PERICYTE CONTRACTION	POLLERES, Marlene
15:40	INACTIVATED SARS-COV-2 VIRAL PARTICLES PROMOTE CILIATION IN TISSUE-ENGINEERED 3D AIRWAY TRI-CULTURES	GONZALEZ-RUBIO, Julian
15:50	A TISSUE ENGINEERING MODEL OF CRANIOSYNOSTOSIS TO IDENTIFY NEW THERAPEUTIC TARGETS THAT ACCELERATE BONE HEALING IN ADULTS	MEYER, Mariangela
16:00	Towards the development of multiaxial loading bioreactor for intervertebral disc studies: validation of an ex vivo organ model and customized sample holder	ŠEĆEROVIĆ, Amra
16:10	Culture of cancer spheroids and evaluation of anti-cancer drugs in 3D-printed miniaturized continuous stirred tank reactors (mCSTRs)	ALVAREZ, Mario
16:20	COLLAGEN-BASED 3D CO-CULTURE MODEL TO INVESTIGATE THE MULTIPLE MYELOMA MICROENVIRONMENT IN BONE MARROW	HERRMANN, Marietta
16:30	PRECLINICAL 3D BIOPRINTED MODEL OF OVARIAN CANCER TUMOR MICROENVIRONMENT TO TEST miRNA-BASED PERSONALIZED THERAPIES	SCOGNAMIGLIO, Chiara
16:40	A BIOPRINTED RHABDOMYOSARCOMA MODEL WITH MACROMOLECULAR CROWDING TO STIMULATE EXTRACELLULAR MATRIX PROTEIN DEPOSITION	D'AGOSTINO, Stefania
16:50	Biological and Mechanical Unique Extracellular Matrix Among Different Subtypes of Dystrophic Epidermolysis Bullosa	MALTA, Mariana D.

S23+S31+S32 Can we bioengineer tissues using artificial cells? + Extracellular vesicles – next generation tool for diagnostics and regenerative medicine + Extracellular vesicles for soft tissue repair - Room: S4 A (15:30 - 17:00)

-Conveners: Anne Des Rieux; Barbara Łukomska; Catherine Le Visage; Ewa Zuba-Surma; Paula Vena

time	title	presenter
15:30	Artificial cells with communicative features, toward hybrid organoids	VAN HEST, Jan
15:50	First steps toward bioprinting artificial cells	DUARTE CAMPOS, Daniela
16:10	Tenocyte conditioned media and its potential applications for immunomodulation.	BYRNE, Amy
16:20	MATRIX-BOUND NANOVESICLES AS SELECTIVE MODULATORS OF THE IMMUNE RESPONSE	CAPELLA-MONSONIS, Hector
16:30	Matrix Bound Nanovesicles as an Immunomodulatory Therapy for Rheumatoid Arthritis	CRUM, Raphael
16:40	ELUCIDATING THE BIOGENESIS OF MATRIX-BOUND NANOVESICLES	DEWEY, Marley
16:50	PLATELET-DERIVED EXTRACELLULAR VESICLES SHOW THERAPEUTIC EFFECTS ON A 3D TENDON DISEASE MODEL	GRAÇA, Ana Luísa

P2 Plenary Session: Ali Khademhosseini (plenary lecture) - Engineering in Precision Medicine - Room: S1 (17:30 - 18:30)

-Conveners: Jos Malda

time	title	presenter
17:30	Engineering in Precision Medicine	KHADEMHOSSEINI, Ali

SYIS Career Panel - Room: S2 (18:30 - 19:30)

Thursday, 30 June 2022

P3 Plenary Session: Shulamit Levenberg (plenary lecture) - Bioprinting 3D vascularized tissue flaps - Room: S1 (09:00 - 10:00)

-Conveners: Lorenzo Moroni

time	title	presenter
09:00	Bioprinting 3D vascularized tissue flaps	LEVENBERG, Shulamit

Debate 2: Beyond the promise of Biofabrication: what needs to be done to bring biofabricated substitutes to the clinic? (Prof. Jürgen Groll, Prof. Daniel J Kelly, Prof. Shulamit Levenberg, Prof. Marcy Zenobi-Wong) - Room: S1 (10:00 - 10:30)

-Conveners: Lorenzo Moroni

time	title	presenter
10:00	Beyond the promise of Biofabrication: what needs to be done to bring biofabricated substitutes to the clinic?	GROLL, Jürgen KELLY, Daniel LEVENBERG, Shulamit ZENOBI-WONG, Marcy

S66 Wanted: Dead or Alive? Quantitative microscopy of spheroid and organoid tissues - Room: S4 C (11:00 - 12:30)

-Conveners: Ruslan I. Dmitriev; Michael Monaghan

time	title	presenter
11:00	Intravital multiphoton and higher harmonic generation microscopy for visualizing cellular processes in cancer and tisseu engineering	WEIGELIN, Bettina
11:20	Non-Invasive classification of macrophage polarisation by 2P-FLIM and machine learning	MONAGHAN, Michael
11:40	MONITORING OF LIVE SPHEROID OXYGENATION USING FLUORESCENCE MICROSCOPY AND NANOSENSORS	OKKELMAN, Irina
12:00	INTEGRATED IMAGING AND MODELLING OF ORGANOID AND SPHEROID MORPHOMETRY USING SMART ALGORITHMS	AHLUWALIA, Arti
12:20	EMT transcriptional response are triggered in response to laser photoablation in 3D models of melanoma	RODRIGUES, Daniel

S10-2 Biofabricated Tissues and Organs for Clinical Impact - Room: S1 (11:00 - 12:30)

-Conveners: Laura De Laporte

time	title	presenter
11:00	Weaving a compliant Tissue-Engineered Vascular Graft from Cell-Assembled extracellular Matrix yarn	ROUDIER, Gaëtan
11:10	Exploring shape versatility on all-aqueous processing for cell encapsulation	OLIVEIRA, Mariana B.
11:20	Microfluidic production of immunoprotective enzymatically crosslinked polyethylene glycol-tyramine microgels for beta-cell replacement therapies	ARAÚJO-GOMES, Nuno
11:30	Tissue Engineered Graft from human Adipose-derived Stem Cells for Phalanx Construction in Children with Symbrachydactyly	MOYA, Adrien
11:40	An innovative in vitro gut-on-a-chip model to investigate intestinal microbiota impact on brain functionality	DONNALOJA, Francesca

11:50	Axially vascularized mandibular regeneration, a journey of thousand miles to improve patients' smiles	EWEIDA, Ahmad
12:00	Engineering the Bioartificial Filtration Unit in a Kidney using Polyhydroxyalkanoates	SYED MOHAMED, Syed Mohammad Daniel
12:10	TOWARDS THE DEVELOPMENT OF A GELMA-BASED ORGANOTYPIC HUMAN SKIN MODEL USING A CUSTOM-MADE BIOREACTOR	ELTAYARI, Zahara
12:20	Laser-based subtractive manufacturing for tissue engineering	CRUZ-MOREIRA, Daniela

S05 Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice - Room: S3 A (11:00 - 12:30)

-Conveners: Veronika Hruschka; Mohammad Alkhraisat

time	title	presenter
11:00	Between risk, privacy and magic: regulatory and reimbursement of individual regenerative implants	SEITZ, Daniel
11:20	Medical additive manufacturing: Is it ready for broad clinical use?	MOSCATO, Francesco
11:40	3D BIOPRINTING OF STRUCTURALLY ORGANIZED MENISCUS TISSUE	BARCELÓ, Xavier
11:50	COMPUTATIONAL MODELLING OF MECHANICAL PROPERTIES OF THE SCAFFOLDS PRODUCED BY MELT ELECTROWRITING	ZIELINSKI, Piotr
12:00	Development of an Electroconductive, 3D-Printed Scaffold Designed to Promote Axonal Regrowth After Spinal Cord Injury	LEAHY, Liam M.
12:10	Multi-material 3D printing of ceramics for fabricating bi-phasic implants	SCHWENTENWEIN, Martin
12:20	DESIGN AND EVALUATION OF LATTICE-STRUCTURED MENISCAL IMPLANTS	TUPE, Disha

S39 Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering - Room: S2 (11:00 - 12:30)

-Conveners: Beata Niemczyk-Soczynska; Paweł Sajkiewicz

time	title	presenter
11:00	INJECTABLE AND PHOTOCURABLE AMPHIPHILIC HYBRID NETWORKS: SYNTHESIS APPROACH USING NON-TOXIC CATALYSTS	EL FRAY, Mirosława
11:20	INJECTABLE THERMOSENSITIVE METHYLCELLULOSE/AGAROSE HYDROGEL AS SMART SCAFFOLD FOR TISSUE ENGINEERING APPLICATIONS	NIEMCZYK-SOCZYNSKA, Beata
11:30	ENZYME-CONTROLLED, NUTRITIVE HYDROGEL FOR MESENCHYMAL STROMAL CELL SURVIVAL AND PARACRINE FUNCTIONS	WOSINSKI, Pauline
11:40	ASSESSING EFFICACY OF REGENERATIVE THERAPIES FOR ISCHAEMIC STROKE - A NOVEL APPROACH FOR MORE MEANINGFUL OUTCOMES IN PRECLINICAL MODELS	SAVA, Roxana
11:50	Designing bioinspired medical adhesives from marine biopolymers and Tannic acid	SACRAMENTO, Margarida
12:00	Drug-loaded Alginate microspheres for breast cancer treatment	PITTON, Matteo
12:10	Advanced stem cell therapy for neurodegenerative diseases	SUSANA COSTA MACHADO FERREIRA, Helena
12:20	HA and PRP combinations as “off the shelf“ device for clinical applications	NARDINI, Marta

S60 Tissue engineering and regenerative medicine in Czech Republic - Room: S4 B (11:00 - 12:30)

-Conveners: Ales Hampl; Giancarlo Forte

time	title	presenter
11:00	The molecular basis of pathological mechanosensing in the failing heart	FORTE, Giancarlo
11:20	Unveiling the molecular basis of pathological mechanosensing to counteract diseases	VINARSKY, Vladimir
11:35	AAV-mediated gene therapy for axon regeneration after spinal cord injury.	JENDELOVA, Pavla
11:50	Generation and Characterization of Human iPSC-derived Cardiac Organoids for Translational Medicine	ERGIR, Ece
12:05	Electrospun silica nanofibres as multifunctional substrate for drug delivery and tissue regeneration	RYSOVÁ, Miroslava

S55 REMODELing the Future: next generation of organoid models for biomedicine - Room: S4 A (11:00 - 12:30)

-Conveners: Silvia Maria Mihăilă; Marta Alves Da Silva

time	title	presenter
11:00	Bioengineering vascularized microtissues	BARRIAS, Cristina
11:20	TBA	RANGA, Adrian
11:40	Combining cholangiocarcinoma organoids and decellularized liver scaffolds unveils microenvironment-dependent extracellular matrix remodeling	VAN TIENDEREN, Gilles
11:50	Microengineered System to Generate the Functional Inner Ear Organoids with Enhanced Uniformity and Maturity	PARK, Sunho
12:00	Synthetic supramolecular hydrogels for the 3D culture of kidney epithelial cells and intestinal organoids	RIJNS, Laura
12:10	Bile duct on a chip: engineering a microfluidic platform for studying biliary epithelium in a dish	WILLEMSE, Jorke
12:20	Differentially expressed microRNAs during endochondral differentiation of human bone marrow derived mesenchymal stromal cells to identify possible biomarkers for non-union fractures	BREULMANN, Franziska

S52 Perspectives For Future Innovation in Tendon repair (P4 FIT) - Room: S3 B (11:00 - 12:30)

-Conveners: Giovanna Della Porta ; Nicholas Forsyth

time	title	presenter
11:00	Advances in bioactive materials for tendon repair	BOCCACCINI, Aldo
11:20	Epithelial-to-mesenchymal transition for tendon regenerative medicine strategies	BARBONI, Barbara
11:40	New tools in tendon tissue engineering	GOMES, Manuela E.
12:00	MiRNAs As Potential Regulators Of Enthesis Healing In A Rodent Injury Model	PENICHE SILVA, Carlos Julio
12:10	MULTIMATERIAL AND MULTISCALE SCAFFOLD FOR TENDON/LIGAMENT REGENERATION	MICALIZZI, Simone
12:20	Development of lipid-polymer hybrid nanoparticles for tendon regeneration	LÓPEZ CERDÁ, Sandra

S53 Prospects and Challenges in Biological Therapies for Tendon Regeneration - Room: S4 B (13:30 - 15:00)

-Conveners: Dimitrios I. Zeugolis; Manuela E. Gomes; Mohammad El Khatib

time title presenter

13:30	What influences tendon biology?	WILDEMANN, Britt
13:50	Inflammation – a Core Feature of Tendinopathies	TRAWEGER, Andreas
14:10	INVESTIGATING INFLAMMATION IN TENDINOPATHY: HOW CAN STEM CELLS HELP US?	SMITH, Emily
14:20	MAGNETIC NANOPARTICLE-MEDIATED ORIENTATION OF COLLAGEN HYDROGELS FOR IN VITRO MODELLING AND REGENERATIVE THERAPIES	WRIGHT, Abigail
14:30	Pro-resolving mediators in rotator cuff tendinopathy: how is the bursa involved?	KLATTE-SCHULZ, Franka
14:40	HUMAN 3D TENDON-ON-CHIP MODEL TO INTERROGATE THE MULTICELLULAR CROSSTALK IN TENDINOPATHY	BAKHT, Syeda Mahwish

S57 Supramolecular synthetic scaffolds: from concept to design and application - Room: S2 (13:30 - 15:00)

-Conveners: Alberto Saiani; Dammy Olayanju

time	title	presenter
13:30	Supramolecular biomaterials for engineering the cell-material interface – from design to screening	DANKERS, Patricia
13:50	Novel insights into the origin of my-fibroblasts using iPSC derived kidney organoids maintained in user defined self-assembling peptide hydrogels	CREAN, John
14:10	IMPROVED GUANOSINE-BASED BIOINKS FOR SOFT TISSUE RECONSTRUCTIONS	GODOY GALLARDO, Maria
14:20	Where are all the electrospun medical devices? – Case studies of product development from an industry perspective	DUCKWORTH, John
14:30	DEVELOPMENT OF MULTIFUNCTIONAL ANTIMICROBIAL SUPRAMOLECULAR BIOMATERIALS	RIOOL, Martijn
14:40	TISSUE ENGINEERING THE OESOPHAGUS: PROOF-OF-CONCEPT	RAI, Nischal
14:50	DESIGN OF 3D PRINTABLE SUPRAMOLECULAR SELF-ASSEMBLING β-SHEET PEPTIDE-HYALURONIC ACID HYDROGELS WITH IMMUNOMODULATORY PROPERTIES	WYCHOWANIEC, Jacek K.

S26 Combined Korea-EU Symposium: "Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells" - Room: S4 C (13:30 - 15:00)

-Conveners: Gunil Im

time	title	presenter
13:30	Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells	IM, Gunil
13:50	Adipose-derived cells for bone regeneration: Bone (pre)fabrication, developmental engineering and vascularization strategies	SCHERBERICH, Arnaud
14:10	ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION SHOWS SUPERIOR OSTEOGENIC DIFFERENTIATION COMPARED TO DONOR-MATCHED MESENCHYMAL STROMAL CELLS	HUSCH, Johanna
14:20	Influence of Dexamethasone on the Interaction Between Glucocorticoid Receptor and SOX9: a Molecular Dynamics Study	STOJCESKI, Filip
14:30	NMR-BASED METABOLOMIC ANALYSIS OF ENDO- AND EXOMETABOLOME ADAPTATIONS THROUGHOUT OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS	BISPO, Daniela S. C.

14:40	UNVEILING LIPID METABOLISM UNDERLYING AGING AND OSTEOGENESIS OF MESENCHYMAL STEM CELLS THROUGH 1H-NMR METABOLOMICS	JESUS, Catarina S. H.
14:50	CONVERGENCE OF SCAFFOLD-GUIDED BONE REGENERATION PRINCIPLES AND MICROVASCULAR TISSUE TRANSFER SURGERY	HUTMACHER, Dietmar W.

S65-1 Vascularization for Tissue Engineering and Regenerative Medicine - Room: S1 (13:30 - 15:00)

-Conveners: Zygmunt Pojda

time	title	presenter
13:30	Therapeutic vascularization in regenerative medicine	BANFI, Andrea
13:50	ENGINEERING HIGH DENSITY CAPILLARY-LIKE NETWORKS USING MICROPOROUS ANNEALED PARTICLE TISSUES	SCHOT, Maik
14:00	SEMAPHORIN3A COUPLES OSTEOGENESIS AND ANGIOGENESIS IN TISSUE-ENGINEERED OSTEOGENIC GRAFTS	GROSSO, Andrea
14:10	A BIOARTIFICIAL FIBRIN-BASED VASCULAR PROSTHESIS WITH A PRE-VASCULARIZED TUNICA ADVENTITIA	ZIPPUSCH, Sarah
14:20	LARGE SCALE FIBRIN-BASED TISSUE CONSTRUCTS SHOW CAPILLARIZATION UPON PERFUSION	ZIPPUSCH, Sarah
14:30	RESET ENDOTHELIAL CELLS PROMOTE FETAL HEPATOCYTE MATURATION IN A 3D ORGANOTYPIC ENVIRONMENT	CACIOLLI, Lorenzo
14:40	THE USE OF HUMAN SKELETAL MUSCLE MICROVASCULAR ENDOTHELIAL CELLS IN SKELETAL MUSCLE TISSUE ENGINEERING: FROM CELL ISOLATION TO IN VITRO PRE-VASCULARIZATION	WÜST, Rebecca
14:50	CELL SHEET-BASED SKIN SUBSTITUTE TO MODULATE VASCULATURE AND INVESTIGATE WOUND-HEALING ASSOCIATED ANGIOGENESIS	MULLER, Laurent

S67 We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration - Room: S4 A (13:30 - 15:00)

-Conveners: Marianna Tryfonidou; Lizette Utomo

time	title	presenter
13:30	A biomimetic approach to regenerate a functional NP tissue in the degenerating intervertebral disc.	ITO, Keita
13:50	Development of advanced regenerative approaches for disc degeneration - consideration of the degenerate niche	LE MAITRE, Christine
14:10	TARGETED PROTEOMIC ANALYSIS TO EXPLORE THE ANTI-INFLAMMATORY EFFECTS OF NOTOCHORDAL-CELL DERIVED MATRIX	LAAGLAND, Lisanne
14:20	MODIC CHANGES CORRELATE WITH ENDPLATE AND VERTEBRAL BONE PATHOLOGIES IN DOGS	BACH, Frances
14:30	Directed differentiation of induced pluripotent stem cells to notochordal-like cells by combinatorial transcription factors activation	TONG, Xiaole
14:40	Tuning the Physical Properties of Collagen/Hyaluronan Hydrogels to favor Mesenchymal Stem Cells Differentiation into NP Cells: A Step forwards Intervertebral Disc Regeneration	HELARY, Christophe
14:50	Proteomic characterisation of foetal notochordal cells to inform intervertebral disc development and stem cell differentiation	RICHARDSON, Stephen

S63 Towards automated technologies for organoid-based tissue biomanufacturing - Room: S3 B (13:30 - 15:00)

-Conveners: Ioannis Papantoniou

time	title	presenter
13:30	Predictive analysis of cardiac microtissue manufacturing by monitoring metabolic CQAs	PALECEK, Sean
13:50	The role of automated bioprocessing within ATMP development and production	DELAHAYE, MIchael
14:10	AUTOMATED MANUFACTURING OF MICROTISSUE BASED OSTEOCHONDRAL IMPLANTS: THE »JOINTPROMISE« PLATFORM	KRIEGER, Judith
14:20	CARTILAGINOUS MICROTISSUES MERGED WITH TAILORED MELT ELECTROWRITTEN MESHES RESULT IN BONE FORMING BIOHYBRIDS	NILSSON HALL, Gabriella
14:30	Laser Assisted Bioprinting for spheroid-based tissue manufacturing	GUILLEMOT, Fabien
14:40	DEVELOPMENT OF A ROBOTICS-DRIVEN BIOMANUFACTURING PROCESS FOR CARTILAGINOUS SPHEROIDS	DECOENE, Isaak
14:50	Stirred culture promotes chondrogenic hypertrophy of cartilaginous microtissues through exposure to intermittent shear stress	LOVERDOU, Niki

S59+S18 The role of multifunctional nanomaterials in new tissue regeneration strategies + Biomedical applications of MXene based next generation nanomaterials - Room: S3 A (13:30 - 15:00)

-Conveners: Aleksandra Benko; Lucia Gemma Delogu; Sanjiv Dhingra

time	title	presenter
13:30	Nanomedicine: Having External Control of Tissue Engineered Materials After Implantation	WEBSTER, Thomas
13:50	The role of multifunctional nanomaterials in new tissue regeneration strategies	REILLY, Gwendolen
14:10	Carbon nanotubes as effective modulators of cellular reactions in various tissue regeneration strategies	BENKO, Aleksandra
14:20	SPATIALLY RESOLVED MONITORING OF IN VITRO AND IN VIVO DEGRADATION IN CARDIOVASCULAR IN SITU TISSUE ENGINEERING	MARZI, Julia
14:30	SMART TANTALUM CARBIDE MXENE QUANTUM DOTS FOR TREATMENT OF ALLOGRAFT VASCULOPATHY	YAN, Weiang
14:40	AEROSOL-JET PRINTING ENABLES HIGH-RESOLUTION TI3C2 MXENE PRINTED ELECTRODES ON A PTFE STRUCTURE FOR NEURAL STIMULATION	GUTIERREZ GONZALEZ, Javier
14:50	THE IMPACT OF PRIMARY AND SECONDARY FIBERS MORPHOLOGY ON REGENERATIVE AND OPTICAL PROPERTIES OF ELECTROSPUN CORNEA IMPLANT	KURPANIK, Roksana

S58 TERMIS-EU SYIS and yESAO joint symposium - Room: S4 C (15:30 - 17:00)

-Conveners: Yi-tung Lu; Zuzana Koci; Lisanne Laagland

time	title	presenter
15:30	Deciphering endochondral ossification to engineer bone: new opportunities for tissue regeneration and disease modelling	LOLLI, Andrea
15:50	Engineering bioactive surface coatings for programming cell behavior towards osteogenic differentiation	GROTH, Thomas
16:10	Nanoengineered Mechanically Robust Bioactive Particles Disseminated in Chitosan/Collagen Matrix for Osteoporotic Bone Treatment	KAUR, Kulwinder

16:20	The differential response of human macrophages to 3D printed titanium antibacterial implants does not affect the osteogenic differentiation of hMSCs	GARMENDIA URDALLETA, Amaia

S45 Nature bioinspired biomaterials and strategies for TERM - Room: S3 A (15:30 - 17:00)

-Conveners: Thomas Groth; Nuno Neves

time	title	presenter
15:30	TBA	REIS, Rui
15:50	CONDUCTIVE HYDROGEL NANOCOMPOSITE-BASED NEURAL INTERFACE FOR IN VIVO RECORDING OF BRAIN CORTEX SIGNALS	RINOLDI, Chiara
16:00	Bio-engineering of a Xenogeneic Vascularized Endocrine Pancreas (VEP) for Type 1 Diabetes	CITRO, Antonio
16:10	4D bioprinting of a dynamic multi-material scaffold for in vitro modeling of neural tube development	DE MARIA, Carmelo
16:20	Electrospinning and Metal Stents – A Good Fit?	KANARI, Konstantina
16:30	From protein-based liquified microcapsules to bone tissue micro-units	R. PINHO, Ana
16:40	Curvature-driven cell suturing controls cell organization and tissue formation inside porous biomaterials	HERRERA, Aaron
16:50	ENGINEERING FUNCTIONAL MICROVASCULARIZED SKELETAL MUSCLE TISSUE EQUIVALENTS VIA MICROFLUIDIC-ASSISTED 3D WET-SPINNING AND MICROVASCULAR SEEDS	PRESUTTI, Dario

S21+S44 Biophysical Therapies - External energy to push internal regeneration + Nano Magnetic platforms - an attractive opportunity for advancing TERM products to the clinic - Room: S4 B (15:30 - 17:00)

-Conveners: Paul Slezak; Peter Dungel; Alicia El Haj; Luminita Labusca

time	title	presenter
15:30	Leveraging Physical Limitations to Expand Shockwave Therapy to Novel Indications	SLEZAK, Cyrill
15:50	ANTIMICROBIAL EFFECTS OF BLUE LIGHT AND RESISTANCE DEVELOPMENT	METZGER, Magdalena
16:00	HUMAN MESENCHYMAL STEM CELLS AND NANOMAGNETIC MATERIALS FOR REGENERATIVE MEDICINE	LABUSCA, Luminita
16:10	A SIMPLIFIED PROTOCOL FOR PREPARATION OF CELL BASED BIOLOGICAL SAMPLES FOR OBSERVING NANOMATERIAL SURFACE ADHERENCE USING SCANNING ELECTRON MICROSCOPY IMAGING	MINUTI, Anca
16:20	Magnetic Nanocarpets based Non-invasive Modulation of Mechanosensitive Ion-channels for Enhanced Osteogenesis	RAJAN UNNITHAN, Afeesh
16:30	Modulating macrophage phenotypes via immune-switch magnetic nanoparticles	ALMEIDA, Ana F.
16:40	Magnetically miRNA-based guidance of macrophage functions	ALMEIDA, Ana F.

S40 Injectable scaffolds in tissue engineering - Room: S2 (15:30 - 17:00)

-Conveners: Qian Xu; Wenxin Wang; Beata Niemczyk-Soczynska

time	title	presenter
15:30	Scaffolds for the Delivery of Gene Therapeutics for Enhanced Tissue Repair	O'BRIEN, Fergal
15:50	Injectable hydrogels for joint regeneration	LE VISAGE, Catherine

time	title	presenter
16:10	LOW-INTENSITY PULSED ULTRASOUND DIRECT CHONDROGENIC DIFFERENTIATION OF ADIPOSE-STROMAL CELLS IN 3D PIEZOELECTRIC HYDROGELS	MANFERDINI, Cristina
16:20	Characterization and molecular imaging of a biohybrid tissue engineered vascular graft	RANJAN MOHAPATRA, Saurav
16:30	Designing injectable peptide-based hydrogels for TERM applications	SAIANI, Alberto
16:40	LIVER-SPECIFIC LIGAND-CONJUGATED MICROPARTICLES FOR TARGETED ISLET TRANSPLANTATION	LEE, I-ning
16:50	An Injectable Hydrogel from a Hydrophobically Modified Collagen for the Encapsulation and Delivery of Fetal Cardiac MSCs	JAMADI KHIABANI, Mahsa

S42 Microphysiological models as powerful preclinical tools - Room: S3 B (15:30 - 17:00)

-Conveners: Ozlem Yesil-Celiktas

time	title	presenter
15:30	Microengineering 3D perfusion networks for human liver tissue models	LARSEN, Niels B.
15:50	Design and Fabrication of an organ-on-a-chip technology as a physiologically relevant in vitro model of the outer Blood-Retinal Barrier	VOZZI, Giovanni
16:10	A GUT-BRAIN AXIS PLATFORM TO MODEL BRAIN FLUIDS CLEARANCE IN NEUROINFLAMMATION	PEROTTONI, Simone
16:20	PRELIMINARY DEVELOPMENT OF AN IN VITRO 3D IPSC-BASED LIVER MODEL TO EXPLOIT AN INNOVATIVE LIVER-ON-A-CHIP DEVICE	FANIZZA, Francesca
16:30	A MICROFLUIDIC PLATFORM TO INVESTIGATE THE CROSS-TALK BETWEEN IMMUNE CELLS IN RHEUMATOID ARTHRITIS	PALMA, Cecilia
16:40	A tunable lung physiomimetic stretch system evaluated with precision cut lungs slices and recellularized human lung scaffolds	IBÁÑEZ-FONSECA, Arturo
16:50	Spatio-temporal emergence of multicellular engineered structures as preclinical models	YESIL-CELIKTAS, Ozlem

S65-2 Vascularization for Tissue Engineering and Regenerative Medicine - Room: S1 (15:30 - 17:00)

-Conveners: Arnaud Scherberich

time	title	presenter
15:30	Pro-angiogenic hydrogels from cell-degradable and photo-curable alginate	FERNÁNDEZ-PÉREZ, Julia
15:40	THERAPEUTIC EVALUATION OF α2-ANTIPLASMIN AS A HUMAN-DERIVED SUBSTITUTE TO THE FIBRINOLYSIS INHIBITOR APROTININ IN SURGERY AND REGENERATIVE MEDICINE	BRIQUEZ, Priscilla
15:50	Blood vessel detection algorithm for tissue engineering and quantitative histology	ADAMO, Arianna
16:00	Homing of bone marrow mononuclear cells to axially vascularized tissue engineering constructs	EWEIDA, Ahmad
16:10	THE IMPACT OF ENDOTHELIAL CELL YAP/TAZ ON NEO-ANGIOGENESIS IN BONE HEALING	MEHL, Julia
16:20	HUMAN IPSC BLOOD VESSEL ORGANOIDS AS A SOURCE OF FLOW-ADAPTIVE VASCULAR CELLS FOR TISSUE ENGINEERING OF PERFUSED MACROVASCULAR GRAFTS.	MEIJER, Elana
16:30	THE EFFECT OF CARTILAGE MATURATION AND MINERALISATION ON ANGIOGENESIS DURING ENDOCHONDRAL OSSIFICATION	JI, Encheng

16:40	Towards tissue-specific vascularization of bio-engineered skeletal muscle constructs using autologous skeletal muscle microvascular endothelial cells	TERRIE, Lisanne
16:50	GLUCOSE ENHANCES TRANSPLANTED MESENCHYMAL STROMAL CELLS FUNCTIONS PERTINENT TO ANGIOGENESIS	WOSINSKI, Pauline

S51+S29 Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine + Engineered viscoelasticity in cell and tissue engineering - Room: S4 A (15:30 - 17:00)

-Conveners: Jacek K. Wychowaniec; Aline F. Miller ; João Mano

time	title	presenter
15:30	Dynamic hydrogel design for spatiotemporal control of morphogenesis	BROGUIERE, Nicolas
15:50	Hydrogels that talk to cells when lighted	DEL CAMPO, Aranzazu
16:10	4D Bioprinting of Self-Bending Scaffolds for Articular Cartilage Tissue Engineering Applications	DÍAZ-PAYNO, P.J.
16:20	CLICKABLE DYNAMIC BIOINKS	TOURNIER, Pierre
16:30	WET-SPUN CORE-SHELL HYDROGEL FIBERS FOR MICROVASCULAR TISSUE ENGINEERING	PARADISO, Alessia
16:40	MICROFLUIDIC SPINNING OF HYDROGEL-BASED CORE-SHELL MICROFIBERS FOR THE FABRICATION OF MYOTENDINOUS TISSUE-LIKE CONSTRUCTS	VOLPI, Marina
16:50	Glycopeptide-based supramolecular hydrogels induce differentiation of stem cells into neural lineages	CASTRO, Vânia I. B.

FTERM Panel Discussion - Room: S1 (17:30 - 18:30)

SYIS Green lab Panel Discussion - Room: S2 (18:30 - 19:30)

Friday, 1 July 2022

P4 Plenary Session: Dietmar W. Hutmacher (plenary lecture): Commentatio historica et philologica - Perspectives and Challenges in Regenerative Medicine - Room: S1 (09:00 - 10:00)

-Conveners: Manuela E. Gomes

time	title	presenter
09:00	Commentatio historica et philologica - Perspectives and Challenges in Regenerative Medicine	HUTMACHER, Dietmar W.

Debate 3: Perspectives and Challenges of Tissue engineering and Regenerative Medicine (Prof. Dietmar Hutmacher, Prof. Małgorzata Lewandowska-Szumiel, Prof. Rui L. Reis) - Room: S1 (10:00 - 10:30)

-Conveners: Manuela E. Gomes

time	title	presenter
10:00	Perspectives and Challenges of Tissue engineering and Regenerative Medicine	HUTMACHER, Dietmar W. REIS, Rui L. LEWANDOWSKA-SZUMIEL, Malgorzata

S34 Advanced therapy approaches in tissue engineering - Room: S4 A (11:00 - 12:30)

-Conveners: Irene Lara-Saez; Wenxin Wang; Hector Capella-Monsonis

time	title	presenter
11:00	Non-viral gene delivery platform for topically treating rare genodermatoses	WANG, Wenxin
11:20	Development of collagen-nanohydroxyapatite scaffold platform for dual-delivery of a microRNA-26a mimic and micoroRNA-133a inhibitor for treatment of large volume bone defects	SADOWSKA, Joanna
11:30	CYSTIC FIBROSIS: REGENERATING LUNG EPITHELIAL CELLS FUNCTION WITH NON-VIRAL GENE THERAPY	MANZANARES SANDOVAL, Dario
11:40	NANOPARTICLE-MEDIATED SELECTIVE SFRP-1 SILENCING ENHANCES BONE DENSITY IN VIVO IN OSTEOPOROTIC MICE BY THE STIMULATION OF THE CANONICAL WNT/β-CATENIN PATHWAY	DIAZ-RODRIGUEZ, Patricia
11:50	MicroRNAs and their role in multiple trauma: profiling local and systemic expression levels	VAN GRIENSVEN, Martijn
12:00	Identification of the best manufacturing condition for clinical grade extracellular vesicles (EVs) secreted by induced pluripotent stem cell-derived mesenchymal stem cells for the treatment of osteoarthritis	GENTILI, Chiara
12:10	A 3D model for the survival niche of human long-lived bone marrow plasma cells	UYAR-AYDIN, Zehra
12:20	OPTIMISING MRNA DELIVERY TO MESENCHYMAL STEM CELLS FOR TISSUE ENGINEERING APPLICATIONS	MCCORMICK, Katie

S50 One health, one medicine: What Veterinary regenerative medicine can teach us - Room: S3 B (11:00 - 12:30)

-Conveners: Iris Gerner; Debbie Guest

time	title	presenter
11:00	THE UTILTIY OF EQUINE PLURIPOTENT STEM CELLS FOR THERAPEUTIC USE AND DISEASE MODELLING	GUEST, Debbie

time	title	presenter
11:20	Synovial membrane-derived mesenchymal progenitor cells from osteoarthritic joints in dogs possess lower chondrogenic-, and higher osteogenic capacity compared to normal joints	TEUNISSEN, Michelle
11:30	The cross-talk between the synovial membrane and cartilage in the distracted canine knee joint	TEUNISSEN, Michelle
11:40	Sheep cells as a suitable in vitro tool to evaluate intervertebral disc biotherapies	HUMBERT, Paul
11:50	Phenotypic Characterization of Adipose-Derived MSC based on their Phospholipid Profiles	BURK, Janina
12:00	HOW DO INFLAMMATION, DIFFERENTIATION, AND MHC COMPATIBILITY AFFECT THE IMMUNOGENICITY AND IMMUNOMODULATORY POTENTIAL OF EQUINE MESENCHYMAL STEM CELLS (MSCs)?	CEQUIER SOLER, Alina
12:10	EVs in equine regenerative medicine – challenges and potential therapeutic implications.	GERNER, Iris
12:20	Induction of the senescence phenotype in equine tendon derived cells by dexamethasone	SMITH, Roger K.W.

S46 New developments of regenerative and tissue modeling products - Room: S1 (11:00 - 12:30)

-Conveners: Xanthippi Chatzistavrou; Faleh Marino

time	title	presenter
11:00	Vat-Polymerization Bioprinting for Tissue Fabrication	ZHANG, Yu Shrike
11:20	Leveraging advances in biomaterials and tissue engineering for reparative, regenerative and tissue modelling solutions	ASHAMMAKHI, Nureddin
11:40	A VOCAL WORKOUT: NOVEL BIOREACTOR FOR THE IN VITRO CULTURE OF VOCAL FOLD REPLACEMENT TISSUES	LUENGEN, Anja E.
11:50	THE COMMITMENT PROFILES OF HEMATOPOIETIC AND MESENCHYMAL STROMAL PRECURSORS IN EX VIVO HEMATOPOIETIC MICRO-TISSUES	BURAVKOVA, Ludmila
12:00	Can oral mucosa be used in primary hypospadias surgery in prepubertal boys?	DE GRAAF, Petra
12:10	BIOENGINEERING A NOVEL UV-INDUCED SKIN MODEL TO MIMIC THE EFFECT OF ENVIRONMENTAL STRESSORS EXPOSURE ON SKIN HEALTH	DE LOS SANTOS GOMEZ, Paola
12:20	NEW HYBRID HYDROGELS FOR APPLICATIONS AS BIOINKS IN 3D PRINTING IMPLANTS	CHATZISTAVROU, Xanthippi

S54+S14 Regulation of cell phenotype in osteochondral tissues: towards RNA therapy for bone and cartilage repair + Biological testing of 3D-printed biomaterials – towards updated norms - Room: S2 (11:00 - 12:30)

-Conveners: Eric Farrell; Andrea Lolli; Veronika Hruschka; Daniel Seitz; Marley Dewey

time	title	presenter
11:00	Cartilage and bone regulation by microRNAs	YOUNG, David
11:20	mRNA therapeutics for musculoskeletal tissue healing	ROSADO BALMAYOR, Elizabeth
11:40	Placing a medical devise in the market: a focus perspective on the biological characterization of a medical device	ALKHRAISAT, Mohammad
12:00	CHROMATIN COMPACTION DECREASES CELL ADHESION STRENGTH: AN ANALYSIS BY FLUIDIC FORCE MICROSCOPY	BUISSON, Julie

12:10	Improving chondrogenic potential of mesenchymal stromal cells by siRNA delivery in hydrogels.	DELLA BELLA, Elena
12:20	3D Printing Of Sol-Gel Silica-Based Hybrids For Bone Regeneration	RODRIGUEZ-GONZALEZ, Raquel

S35+S36 Giving meaning to early tissue damage responses in regeneration + Glycomodulation Approaches in Tissue Engineering - Room: S3 A (11:00 - 12:30)

-Conveners: Johannes Grillari; Heinz Redl; Laura Russo; Abhay Pandit

time	title	presenter
11:00	The Zone of Tissue Activation Delineates Immediate and Long-Term Response of Skin to Wounding and Associates with Markers of Senescence and Regeneration	OGRODNIK, Mikolaj
11:20	Endogenous Bioelectric controls of growth and form	MICHAEL, Levin
11:40	Using Supramolecular Biomaterials to Interrogate and Manipulate Galectin-Glycan Interactions	HUDALLA, Greg
12:00	ENHANCING TISSUE REGENERATION BY DELIVERING AN ENGINEERED TREG-DERIVED FACTOR	PIOTTO, Celeste
12:10	ELASTIN-LIKE-RECOMBINAMER CRYOGEL WITH RECOMBINANT GLYCOSAMINOGLYCANS AS A MODULAR PLATFORM FOR REGENERATION	SÖDERLUND, Zackarias
12:20	Guided bone regeneration in osteoporosis by plant-derived nanoparticles	GURZAWSKA-COMIS, Katarzyna

S61 Tissue Engineering in Microgravity for Health in Space and on Earth - Room: S4 B (11:00 - 12:30)

-Conveners: Jeremy Teo

time	title	presenter
11:00	Tissue Density Diminishes the Effects of Simulated Microgravity on Dendritic Cell Immune Potency in vitro	TEO, Jeremy
11:10	3D microenvironment maintains the transcriptome profile of T cells but not B cells in simulated microgravity	ELGINDI, Mei
11:20	Studies of cellular differentiation in simulated microgravity reveal an important role for β-actin in mechanosensing	SAPKOTA, Oscar

Closing Session and Awards - Room: S1 (12:30 - 13:30)

Tuesday, 28th June 2022	
PS01	3D in vitro tissue-engineered cancer/disease models – Session I
PS02	3D in vitro tissue-engineered cancer/disease models – Session II
PS03	3D printing of bionic organs – how far are we from clinical application?
PS06	Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective
PS07	Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration
PS08	Antimicrobial biomaterials for bone regeneration
PS09	Biobanking – indispensable support for the development of regenerative medicine
PS12	Biofabrication with light-based technologies and high-definition printing
PS16	Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential
PS17	Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility
PS19	Biomimetic Approaches to Cardiovascular Regeneration: how and why?
PS22	Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine
PS25	Cellular senescence in tissue damage and regeneration
PS33	From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine
PS49	Novel strategies to assess cellular response to biomaterials
PS64	Understanding and preventing early inflammatory events that lead to development of osteoarthritis
PS68	Human brain organoids versus assembloids approach for neurodevelopmental studies

Wednesday, 29th June 2022	
PS10	Biofabricated Tissues and Organs for Clinical Impact
PS11	Biofabrication using extrinsic fields
PS13	Biofunctionalized surfaces for cellular and tissue engineering
PS15	Biologically inspired and Engineered disease models
PS20	Biomimetic in vitro models for bone regeneration and cancer pathologies
PS24	Cell-rich constructs for tissue engineering
PS27	Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses
PS28	Emerging and future technologies for peripheral nerve regeneration
PS30	European regional platforms for TERM – Update
PS31	Extracellular vesicles – next generation tool for diagnostics and regenerative medicine
PS32	Extracellular vesicles for soft tissue repair
PS37	Human Organoids for Musculoskeletal Tissues
PS38	Injectable biomaterials for cell-instructive matrix cues
PS41	Mesenchymal Stem / Stromal Cells – from basic research through clinical studies to registered products
PS43	Multifunctional biomaterials supporting bone regeneration
PS47	New insights underlying mesenchymal stem cell-mediated bone regeneration
PS48	Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy
PS56	Skin wound healing in 2022: where basic science meets clinical needs
PS62	Tissue regeneration by integration of bioinspired materials

Thursday, 30th June 2022	
PS05	Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice
PS14	Biological testing of 3D-printed biomaterials – towards updated norms
PS18	Biomedical applications of MXene based next generation nanomaterials
PS21	Biophysical Therapies – External energy to push internal regeneration
PS26	Combined Korea-EU Symposium: "Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells"
PS29	Engineered viscoelasticity in cell and tissue engineering
PS34	Advanced therapy approaches in tissue engineering
PS35	Giving meaning to early tissue damage responses in regeneration
PS36	Glycomodulation Approaches in Tissue Engineering
PS39	Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering
PS40	Injectable scaffolds in tissue engineering
PS42	Microphysiological models as powerful preclinical tools
PS45	Nature bioinspired biomaterials and strategies for TERM
PS46	New developments of regenerative and tissue modeling products
PS50	One health, one medicine: What Veterinary regenerative medicine can teach us
PS51	Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine
PS52	Perspectives For Future Innovation in Tendon repair (P4 FIT)
PS53	Prospects and Challenges in Biological Therapies for Tendon Regeneration
PS54	Regulation of cell phenotype in osteochondral tissues: towards RNA therapy for bone and cartilage repair
PS55	REMODELing the Future: next generation of organoid models for biomedicine
PS57	Supramolecular synthetic scaffolds: from concept to design and application
PS58	TERMIS-EU SYIS and yESAO joint symposium
PS59	The role of multifunctional nanomaterials in new tissue regeneration strategies
PS60	Tissue engineering and regenerative medicine in Czech Republic
PS61	Tissue Engineering in Microgravity for Health in Space and on Earth
PS63	Towards automated technologies for organoid-based tissue biomanufacturing
PS65	Vascularization for Tissue Engineering and Regenerative Medicine
PS66	Wanted: Dead or Alive? Quantitative microscopy of spheroid and organoid tissues
PS67	We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration

BOARD #	POSTER ABSTRACT TITLE	PRESENTER
	PS01 3D IN VITRO TISSUE-ENGINEERED CANCER/DISEASE MODELS – SESSION I	
PS01.1	3D ADIPOSE-LIKE TISSUE ANALOGUES BEARING INFLAMED AND HYPERTROPHIED ADIPOCYTES TO STUDY OBESITY IN VITRO	Alexandra P. Marques
PS01.2	3D PROSTATE CANCER IN VITRO MODELS	Khalsa Alhusaini
PS01.3	A TAILORED BIOREACTOR SYSTEM COMBINED WITH A BIOPRINTED MICROVESSEL SUBSTITUTE ENABLES THE INVESTIGATION OF CHEMOTAXIS IN IN VITRO MODELS	Mattis Wachendörfer
PS01.4	A THREE-DIMENSIONAL DYNAMIC MODEL OF OVARIAN CANCER BY USING A PERFUSION BIOREACTOR	Tali Tavor Re'em
PS01.5	A TISSUE ENGINEERING APPROACH TO STUDY BONE METASTASES IN VIVO	Maria Elisabetta Federica Palamà
PS01.6	ALGINATE MICROFIBERS WITH IMMOBILIZED CANCER CELLS AS A 3D CANCER MODEL FOR ANTICANCER DRUG TESTING	Jelena Petrovic
PS01.7	BIOENGINEERING THE HUMAN BONE NICHE WITH HIGH ADIPOSE CONTENT TO STUDY ADVANCED CANCER IN VITRO AND IN VIVO	Nathalie Bock
PS01.8	BIOMIMETIC THREE-DIMENSIONAL IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER UTILIZING GELMA HYDROGELS	John Saliba
PS01.9	COAXIAL PRINTING OF CONVOLUTED PROXIMAL TUBULE FOR KIDNEY DISEASE MODELLING	Anne Metje Van Genderen
PS01.10	DERIVED TUMOR EXTRACELLULAR MATRIX IS SUITABLE TO 3-DIMENSIONAL CELL GROWTH AND PROVIDE A PROPER ENVIRONMENT FOR TUMOR CELLS	Marta Nardini
PS01.11	DESIGN OF A COMBINED COLLAGEN AND LUNG DECELLULARIZED EXTRACELLULAR MATRIX HYDROGEL FOR THE STUDY OF THE LUNG TUMOR MICROENVIRONMENT (TME)	Lara Milián
PS01.12	DEVELOPING A 3D MODEL OF COLORECTAL CANCER TUMOUR MICROENVIRONMENT	Eileen Reidy
PS01.13	ELUCIDATION OF COLLAGEN FIBRE STRUCTURE IN OSTEOGENESIS IMPERFECTA USING SECOND HARMONIC GENERATION IMAGING ON POLYCAPROLACTONE FIBRES	Gwendolen Reilly
PS01.14	ENGINEERING A BIOMIMETIC THREE-DIMENSIONAL TUBULAR ORGAN-ON-CHIP USING SYNTHETIC BIOPOLYMERS TO MODEL PATHOPHYSIOLOGICAL CONDITIONS	George Deeb
PS01.15	ENGINEERING BIOMIMETIC PATIENT DERIVED RENAL CELL CARCINOMA TUMOUROIDS	Kalliopi Bokea
PS01.16	GLIOMA-ON-A-CHIP PLATFORM	Merve Ustun
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European Chapter of the Tissue Engineering & Regenerative Medicine International Society 2023

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Marianna Tryfonidou

(Regenerative Orthopedics Faculty of Veterinary Medicine, Utrecht University)

Professor Tryfonidou (1973) joined the department of Clinical Sciences of the Faculty of Veterinary Medicine (1998), followed an annual Internship and completed her PhD (2002) Cum Laude. In 2007 she certified as a veterinary surgeon (European College of Veterinary Surgeons (ECVS)). In 2018 she was appointed as Professor Regenerative Orthopedics.
The focus of her research is on understanding the underlying pathophysiology and on developing treatment strategies for back pain and osteoarthritis. Challenges are addressed by combining her clinical background, unique spontaneous diseased canine models with cutting edge biomolecular techniques.
The "Dutch Arthritis Society" (ReumaNederland) recognized the Tryfonidou lab as a Research Centre of Excellence. She has participated in large public-private partnerships (e.g. BioMedical Materials IDiDAS, LSH ArIADNE, AO Spine Research Spine Network) where she coordinated translation of therapeutic strategies, being successful at the cross road of industry, academia, clinicians and patients.
She leads the Horizon 2020 consortium (iPSpine; 2019-2023) bringing a transdisciplinary team of 21 partners together to address the challenges and bottlenecks of iPS-based advanced therapies towards their transition to the clinic. Here, chronic back pain due to intervertebral disc degeneration is employed as a show case. Having demonstrated the ability to develop her own innovative line of research and to act as coach for young researchers, she recently received the prestigious VICI grant in the NWO Talent Programme to develop an nanomedicine-based therapeutic approach.

Dietmar W. Hutmacher

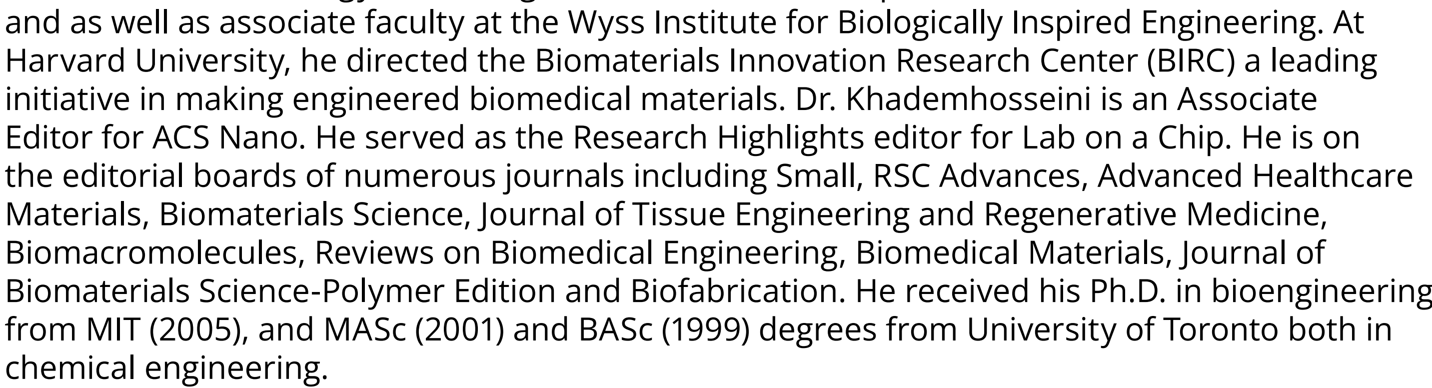
(Max Planck Queensland Centre on the Materials Science for Extracellular Matrices, Queensland University of Technology)

Prof. Dietmar W. Hutmacher is one of the pioneers and world leaders in the field of biomaterials science and TE & RM. The impact of his publications is widely acknowledged through citations and is supported by research funding from both competitive grant schemes and industry collaborations. His research scholarly track record illustrate successful mastery of a major challenge in an interdisciplinary field: the ability to transcend traditional disciplinary boundaries, to initiate and nurture excellent research and educational programs across different disciplines. This was achieved through an interdisciplinary research program via convergence of science & engineering (bioengineering, biomaterials science, computational modelling, chemistry and nanotechnology), the life science disciplines (molecular & cell biology, stem cell research, genomics, proteomics, bioinformatics), and clinical research (orthopedics, plastic and reconstructive surgery, radiology). He is one of the few academics who successfully translated tissue engineering research programs from fundamental research to routine clinical application.

Ali Khademhosseini

(Terasaki Institute for Biomedical Innovation)

Ali Khademhosseini is currently the CEO and Founding Director at the Terasaki Institute for Biomedical Innovation. Previously, he was a Professor of Bioengineering, Chemical Engineering and Radiology at the University of California-Los Angeles (UCLA). He joined UCLA as the Levi Knight Chair in November 2017 from Harvard University where he was Professor at Harvard Medical School (HMS) and faculty at the Harvard-MIT's Division of Health Sciences and Technology (HST), Brigham and Women's Hospital (BWH) and as well as associate faculty at the Wyss Institute for Biologically Inspired Engineering. At Harvard University, he directed the Biomaterials Innovation Research Center (BIRC) a leading initiative in making engineered biomedical materials. Dr. Khademhosseini is an Associate Editor for ACS Nano. He served as the Research Highlights editor for Lab on a Chip. He is on the editorial boards of numerous journals including Small, RSC Advances, Advanced Healthcare Materials, Biomaterials Science, Journal of Tissue Engineering and Regenerative Medicine, Biomacromolecules, Reviews on Biomedical Engineering, Biomedical Materials, Journal of Biomaterials Science-Polymer Edition and Biofabrication. He received his Ph.D. in bioengineering from MIT (2005), and MASc (2001) and BASc (1999) degrees from University of Toronto both in chemical engineering.



Shulamit Levenberg

Faculty of Biomedical Engineering, Technion

Prof. Shulamit Levenberg is the head of the Stem Cell and Tissue Engineering lab at the Technion Faculty of Biomedical Engineering. She serves as the director of the Technion Center for 3D Bioprinting and the Schneur Center for Diabetes Research. Prof. Levenberg received numerous prizes for her work on engineering vascularized tissue constructs for regenerative medicine. The most recent ones include the Rappaport Prize for Excellence in Biomedical Sciences, the Michael Bruno Memorial Award and a Medal of Distinction from

the Peres Center for Peace and Innovation. Prof. Levenberg is founder and CSO of three start-up companies in the areas of spinal cord regeneration, cultivated meat and nanoliter diagnostic arrays. She is a former member of the Israel National Council for Bioethics and is actively involved in training young scientists.

Gerjo van Osch

(Erasmus University Medical Center in Rotterdam)

Gerjo van Osch studied Medical Biology and received her PhD in 1994 for research on animal models for osteoarthritis. As postdoc she became involved in cartilage tissue engineering and moved to Erasmus MC Rotterdam where she has set up a multi-disciplinary research group.
Her research focusses on cellular mechanisms in cartilage repair and involves tissue engineering of the osteochondral unit as well as disease models for osteoarthritis. Since 2012 Gerjo holds a chair in Connective Tissue Regeneration at Erasmus MC and since 2020 she was co-appointed as Medical Delta Professor at Delft University of Technology. Gerjo has supervised more than 40 PhD students and postdocs and co-authored over 250 publications.
She has been involved as PI and coordinator in (inter)national research projects and active in various committees and boards. She is currently vice-chair of the Gordon Conference Cartilage Biology and Pathology and past-chair of TERMIS-EU.

Adam Maciejewski MD, PhD

Professor of Surgery, Godina Fellow Maria Sklodowska-Curie National Research Institute of Oncology

Professor Maciejewski graduated from Medical University of Silesia and underwent general, oncologic and plastic surgery training. Head of the oncological and reconstructive surgery clinic and the leader and founder of the reconstructive team as well as the face and neck transplant team. Three-time ASRM best case winner. In 2020, Prof. Maciejewski had the honor being the American Society for Reconstructive Microsurgery Godina Traveling Fellow in recognition of his accomplishments in the field of reconstructive microsurgery. With his team, he performed the first face transplant in Poland and the first immediate face transplant in the world. He also performed first complex allotransplantation of neck organs in the world.
Dr. Maciejewski has served as visiting professor and guest faculty at numerous national and international congresses and universities (MD Anderson, The Buncke Clinic). He is also the recipient of numerous fellowship and resident teaching awards.

Mohammad Alkhraisat

(BTI Biotechnology Institute), Eduardo Anitua (BTI Biotechnology Institute)

Dr. Mohammad Hamdan Alkhraisat holds a degree in dentistry from the University of Jordan (1996-2001). He also has a diploma in Oral Implantology from the University of Sevilla, a specialist degree in Oral Medicine from the Complutense University of Madrid and a doctorate in Biomaterials from the Complutense University of Madrid. He also has the quality mention of "European Doctorate". Additionally, Dr. Alkhraisat has a Master's degree in Biophysics from the Universidad del Autónoma de Madrid. He is part of the teaching team of two of Master's degrees offered by the University Institute for Regenerative Medicine and Oral Implantology- UIRMI (UPV/EHU- Eduardo Anitua Foundation). He has also supervised several Doctoral Theses at the Universidad Complutense de Madrid, Universidad Rey Juan Carlos and Universidad Europea de Madrid. He is the current Secretary of the Governing Council of the University Institute for Regenerative Medicine and Oral Implantology - UIRMI (UPV/EHU- Eduardo Anitua Foundation). He has also been concerned with acquiring training in Good Laboratory Practice, Good Clinical Practice, clinical evaluations (under the MEDEV and MDR regulations) and FDA 510 (K).
During the years 2004-2010 he was part of the research group "Bioactive Nanostructured Materials" directed by Prof. Enrique López-Cabarcos from the Complutense University of Madrid. During this time he was also a visiting researcher in several European research centers. From the end of 2010 until 2018 he worked as a senior researcher and responsible of the biomaterials area at BTI Biotechnology Institute I mas D. Since 2019 he is the coordinator of the Regenerative Medicine Department at BTI Biotechnology Institute I mas D.
His professional career focused on the development of new biomaterials and in particular bone graft substitutes based on calcium phosphates and autologous biomaterials derived from blood. His research and development interests include synthesis, characterization, intelligent drug delivery systems and the application of these materials (methods and concepts) in the clinic. His way of working would include, thanks to his background in basic and clinical sciences, the understanding of clinical, regulatory and technical limitations of regenerative medicine. He has also published and presented widely in his field (> 130 publications). He is a scientific reviewer of numerous indexed scientific journals with high impact factor.

Niyaz Alsharabi

(University of Bergen/Department of Clinical Dentistry, Center of Translational Oral Research (TOR) – Tissue Engineering Group, Faculty of Medicine City)

CV review:
I am a trained dentist and in 2016 I took a doctorate at the Department of Clinical Dentistry (IKO) at the University of Bergen (UiB), Norway, on the topic of mesenchymal stem cells (MSC)-secretome in the healing of dental tissue damage. After my PhD, I worked until 2018 as a volunteer stem cell researcher at the Princess Al Jawhara Center for Molecular Medicine in Bahrain. From 2019 until now, I work as a researcher/Postdoctoral Fellow at IKO, and my area of interest is the application of MSC and their secretome, including exosomes and extracellular vesicles (EVs), in the treatment of bone tissue defects, pulp regeneration and Multiple sclerosis.
In 2020, I received an external grant from the ITI Research Foundation (project no. 1417_2019). I was also a co-applicant on two large projects that were awarded funding from Helse-vest (2020) and the Mohn Foundation (2021). Recently, I received a Meltzer grant from Norway (2022) for a small project.

I am also involved in several interdisciplinary projects, as follows:
1- Proteomics evaluation of MSC-secretome from dental pulp stem cells
2- MSC-secretome: anti-inflammatory potential in vitro and on bone regeneration in vivo
3- Development of 3D-printed engineered nanocomposite templates for use in stem cell-mediated bone regeneration
4- Effect of umbilical cord stem cells and their secretome on the development of in vitro lung organoids
5- Treatment of Multiple Sclerosis with MSC and secretome in an animal model
6- Effect of EVs from immune cells on MSC and vis versa.
7. Optimization of MSC transport medium for cell therapy applications
8- Encapsulation of MSC-secretome for bone regeneration
Most important scientific publications in the last 5 years:
A- As the last author
1- R Saleem, S Mohamed-Ahmed, R Elnour, E Berggreen, K Mustafa, N Al-Sharabi. Conditioned Medium from Bone Marrow Mesenchymal Stem Cells Restored Oxidative Stress-Related Impaired Osteogenic Differentiation. International Journal of Molecular Sciences 22 (24), 13458. 2021. Impact factor: 5.6.
B- As a co-author
1- N Rana, S Suliman, N Al-Sharabi, K Mustafa. Extracellular Vesicles Derived from Primed Mesenchymal Stromal Cells Loaded on Biphasic Calcium Phosphate Biomaterial Exhibit Enhanced Macrophage Polarization. Cells 11 (3), 470, 2022. Impact factor: 6.5
2- G Al-Qadhi, Aboushady, N Al-Sharabi. The gingiva from the tissue surrounding the bone to the tissue regenerating the bone: a systematic review of the osteogenic capacity of gingival mesenchymal stem cells. Stem Cells International, 2021. Impact factor: 5.443
3- A. Omer, N Al-Sharabi, Y Qiu, Y Xue, Y Li, M Fujio, K Mustafa, Z Xing. Biological responses of dental pulp cells to surfaces modified by collagen 1 and fibronectin. Journal of Biomedical Materials Research Part A 108 (6), 1369-1379, 1, 2020. Impact factor: 4.396
4- M Fujio, Z Xing, N Sharabi, Y Xue, A Yamamoto, H Hibi, M Ueda, Fristad Conditioned media from hypoxic-cultured human dental pulp cells promotes bone healing during distraction osteogenesis. Journal of tissue engineering and regenerative medicine, 11 (7), 2116-2126, 2017. Impact factor: 3.963

Maria Grazia Raucci

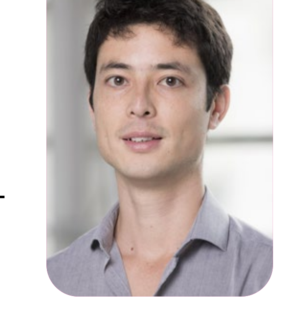
(Institute of Polymers, Composites and Biomaterials/National Research Council)

Luigi Ambrosio is Director of Institute of Polymer, Composites & Biomaterials, National Research Council, Naples, Italy. He received the doctoral degree in Chemical Engineering (1982) from University of Naples “Federico II’. Qualified Full Professor in Bioengineering and in Materials Science and Technology.
Director of Institute of Composites and Biomedical Materials, National Research Council, Naples, Italy (2008-2012).
President of the European Society of Biomaterials (2007-2013), Past President (2013-2017), Honorary Member (since 2018).
Director of Chemical Sciences & Materials Technology Department, National Research Council, Rome, Italy (2011-2017).
Member of the High Level Group on Key Enabling Technologies, European Commission (2010-2015). Member of the International Advisor Board of Sichuan University and Co-Director of MPBRC, SCU-CNR Joint Research Centre. (since 2016).
He is recipient of the “G. Winter Award”, for the high worldwide contribution to the Biomaterials

Science, European Society for Biomaterials (March 2015), China-Italy Science and Technology Innovation Cooperation Contribution Award from China-Italy Technology Transfer Centre, Nov. 2017, Beijing, China.
He has been nominated Fellow of American Institute for Medical and Biological Engineering (2001), Fellow of Biomaterials Science and Engineering (2004) and Fellow of the European Alliance for Medical and Biomedical Engineering & Science (2018) and Member of the European Academy of Science (2019).
Editor-in-Chief of Journal of Materials Science: Materials in Medicine (since 2017).
Research interests include design and characterisation of polymers and composites for medical applications and tissue engineering, structural properties of natural tissue, properties and processing of polymers and composites and nanostructures, hydrogels and biodegradable polymers, additive technologies.
Publications include over 320 papers on international scientific journals and book, 18 patents, over 170 invited lectures and over 500 presentations at international and national conferences.

James Armstrong

(University of Bristol)



Dr James Armstrong leads a research group in Translational Health Sciences based in Bristol Medical School at the University of Bristol. Since graduating from his PhD in 2015, he has been supported by three personal Fellowship awards: Arthritis Research UK (2015-2018), the Medical Research Council (2018-2021), and now a prestigious UKRI Future Leaders Fellowship (2021-2028). These major funding awards have enabled him to lead a programme of highly interdisciplinary and collaborative research focussed on using biomaterials, nanomaterials, and remote fields to engineer artificial tissues with structural and functional complexity. He is now expanding his research interests into engineering stem-cell-derived organoids while also seeking to translate his technologies for applied biological modelling and regenerative medicine.

Matthew Baker

(Maastricht University)

Matthew Baker is an Assistant Professor at Maastricht University, and the group leader of the BioMatt group. He is a chemist by training, and the group takes a molecular view of materials design. Moving from South Carolina, to Florida, to the Netherlands, international research is ingrained in his approach. As a result, our group aims to keep a mix of nationalities, backgrounds, and expertise.
Dr. Matthew (Matt) Baker received his B.S. in chemistry (2006) at Clemson University and worked shortly for Tetramer Technologies, LLC. Afterwards, he obtained his PhD in 2012 in Physical Organic Chemistry under the guidance of Ronald K. Castellano at the University of Florida. He then moved to Eindhoven University of Technology to design and characterize water soluble supramolecular polymers under guidance of Prof. E. W. Meijer. During his time at TU/e, he starting thinking of cells by creating supramolecular hydrogelators for extracellular matrix (ECM) mimics.
In May 2015, Matthew Baker joined the MERLN institute as a researcher, while also starting a group to explore the utility of dynamic interactions in biomaterials. In 2017, he was promoted

to Assistant Professor, founding a group to rationally design, synthesize, and characterize biomaterials based around stimuli-responsive and dynamic interactions. Currently, he serves as the group's biggest fan, and aims to train scientists at the interface of Chemistry and Bioengineering. He loves NMR, maybe a bit too much for the group's liking.

Andrea Banfi

(Basel University Hospital)

Dr. Andrea Banfi graduated in Medicine (1996) and specialized in Clinical Oncology (2000) at the University of Genoa (Italy), studying the biology of bone marrow mesenchymal stem cells and their use for bone tissue engineering in the Cancedda lab. He then worked as a postdoc at Stanford University (USA) in the Blau lab, focusing on cell-based gene delivery for therapeutic angiogenesis. In 2004 he became Research Scientist at Stanford with a Career Development Award by the American Heart Association. In 2005 he moved to Basel (Switzerland), where he directs the group of Cell and Gene Therapy at the University Hospital, in the Department of Biomedicine. Research in the group is funded by several Swiss and European agencies. He is author of about 70 peer-reviewed publications, with about 4'000 citations (h-index: 31) and is also Chief Specialty Editor at Frontiers in Tissue Engineering and Regenerative Medicine – Preclinical Cell and Gene Therapy.
The goal of his group is to promote vascular growth for tissue repair, combining expertise on mesenchymal progenitor cell biology and vascular biology. His research aims at: 1) elucidating the basic mechanisms governing the growth of blood vessels under therapeutically relevant conditions, and 2) translating these concepts into rational regenerative medicine approaches, to restore blood flow in ischemia and to regenerate vascularized tissues, particularly bone. Core competences of the group are the combination of stem cell therapy and gene therapy, as well as engineering of controlled regenerative microenvironments by factor-decorated smart biomaterials.

Barbara Barboni

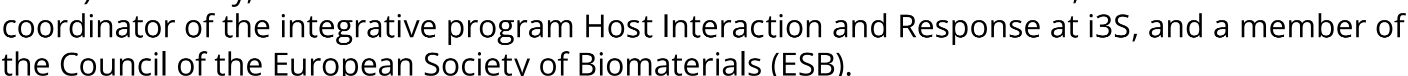
(University of Teramo)

Graduated in Veterinary Medicine and awarded with Ph.D in Neuroendocrinology from University of Bologna. From 2000, she is head of Basic and applied Science Unit of the University of Teramo. Her main research interests are focused on coupling cell/tissue systems models and advanced image analyses to develop biotechnology solution combining both experimental and computational approaches.

Cristina Barrias

(i3S/INEB-Instituto de Investigação e Inovação em Saúde/Instituto de Engenharia Biomédica)

Cristina Barrias is Principal Investigator and Group Leader at i3S/INEB-Institute for Research and Innovation in Health/Instituto de Engenharia Biomédica (University of Porto) and invited Associate Professor at the Institute of Biomedical Sciences Abel Salazar (ICBAS, University of Porto) and at the Instituto Superior de Engenharia do Porto (ISEP, Polytechnic of Porto). Currently, she is also a member of the Board of Directors of INEB, vice-coordinator of the integrative program Host Interaction and Response at i3S, and a member of the Council of the European Society of Biomaterials (ESB).



She is the head of the Bioengineered 3D Microenvironments group at i3S focusing on (micro) tissue engineering strategies for regenerative therapies and in vitro modeling of human tissues/organs. Her group designs customizable, biofunctionalized hydrogel-based 3D matrices and uses biofabrication tools for driving multicellular self-organization into micro-sized building blocks for bottom-up tissue engineering. These 3D microsystems recapitulate complex morphogenetic processes, providing key tools for studying cell-to-cell and cell-matrix crosstalk under physiological and pathological contexts, and for uncovering biochemical and biomechanical regulators of cell behavior. This knowledge is currently being translated into the design of advanced in vitro 3D models of vascularized human tissues/organs and innovative pro-angiogenic cell-based therapies.

Maria Bernabeu

European Molecular Biology Laboratory, Barcelona, Spain

PhD ISGlobal/ Universitat de Barcelona, Spain. 2013
Postdoctoral research at Center for Infectious Disease Research and Seattle Children's Research Institute, Seattle, USA. 2014-2019
Group leader at EMBL from October 2019.
ERC Investigator since 2021.

Aldo Boccaccini

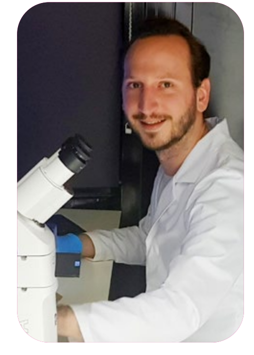
(University of Erlangen-Nuremberg)

Aldo R. Boccaccini is the Head of the Institute of Biomaterials and Professor of Materials Science (Biomaterials) at University of Erlangen-Nuremberg, Germany. He is a visiting professor at Imperial College London, UK. Boccaccini has a Nuclear Engineering degree from Instituto Balseiro, Argentina (1987) and a Doctorate in Engineering Sciences (Dr.-Ing.) from RWTH Aachen University, Germany (1994). He had post-doctoral appointments at the School of Metallurgy and Materials, University of Birmingham, UK (1994-1996), and at the Institute for Mechanics and Materials, University of California, San Diego, USA (1996-1997). He completed the Habilitation in Materials Technology at Ilmenau University of Technology, Germany in 2001. From 2000 to 2009 Boccaccini was Lecturer, Reader and Professor of Materials Science and Engineering at the Department of Materials, Imperial College London, UK. He was the Head of the Department of Materials Science and Engineering at University of Erlangen-

Nuremberg in the period 2017-2019.
The research activities of Prof. Boccaccini are in the field of ceramics, glasses and composites for biomedical, functional and/or structural applications. He is the author or co-author of more than 1000 scientific papers and 25 book chapters. He has co-edited 8 books. His work has been cited more than 53,000 times (h-index = 100, Scopus) and he was included in the "Highly Cited Researchers" lists in 2014 and 2018 (Clarivate). He has been a visiting professor at different universities around the world and has given more than 100 presentations at international conferences (keynote, invited, plenary).
Prof. Boccaccini serves the scientific community in several capacities. He is the vice-president of the Federation of European Materials Societies (FEMS), where he represents the German Materials Society (DGM). He is a member of Council of the European Society for Biomaterials (ESB) (since 2015), being currently the vice-president of ESB. Prof. Boccaccini is the Editor-in-Chief of the journal "Materials Letters" (since 2010). He is a Fellow of four major materials science/technology learning societies, namely: American Ceramic Society, Institute of Materials, Minerals and Mining (UK), European Ceramic Society, and Society of Glass Technology (UK). Prof. Boccaccini has received multiple research awards and honors, including the Materials Prize of the German Materials Society (2015), the Turner Award of International Commission on Glass (2016) and Friedberg Lecture Award (2016) of American Ceramic Society. Boccaccini is also an elected member of the World Academy of Ceramics and of the National Academy of Engineering and Applied Sciences of Germany (acatech). He is an advisor to the Science and Technology Ministry of Argentina. He has served in review panels of several institutions including the European Research Council (ERC) and the German Research Foundation (DFG).

Nicolas Broguiere

(EPFL)



2005-2007 Undergrad studies in Mathematics and Physics, Prépa Champollion, Grenoble, France
2007-2010 Ecole polytechnique, Paris, France, majoring Physics and Biology.
2010-2012 KTH, Stockholm, Sweden, double degree in Biomedical Engineering.
2012-2018 PhD in the Tissue Engineering and Biofabrication Laboratory (Prof. Zenobi-Wong). Thesis title: Extracellular matrices supporting and guiding neurons.
Obtained research award for best PhD thesis by the Swiss Society for Biomaterials and Regenerative Medicine (SSBRM), finalist for best thesis in Materials and Processes (MaP award), ETH medal.
2015-2017 Visiting scientist in the Axonal Regeneration Laboratory (Prof. Bradke). Studying hydrogels for axonal regeneration and spinal cord injury management applications.
2018-now Post-doctoral researcher in the Stem Cell Bioengineering Laboratory (Prof. Lutolf). Developing next generation organoid models of intestinal tissue, neural tissue, early embryonic development, and colorectal cancer.

Guoping Chen

(Research Center of Functional Materials, National Institute for Materials Science)

Prof. Guoping Chen received his Ph.D. from Kyoto University in 1997 majoring in Biomaterials and did postdoctoral research until 2000. He became Researcher in 2000 and Senior Researcher in 2003 at Tissue Engineering Research Center, National Institute for Advanced Industrial Science and Technology, Japan. He moved to Biomaterials Center, National Institute for Materials Science (NIMS) as Senior Researcher in 2004 and was promoted to Group Leader in January, 2007. He was Principal Investigator and Unit Director of Tissue Regeneration Materials Unit from April, 2011 to March, 2015; Principal Investigator, Field Coordinator and Unit Director of International Center for Materials Nanoarchitectonics, NIMS from April, 2015 to March, 2017. He is also Professor of Department of Materials Science and Engineering, Graduate School of Pure and Applied Science, University of Tsukuba, Japan. His research interests include tissue engineering and regenerative medicine, polymeric porous scaffolds, photothermal scaffold, nanobiomaterials, biomimetic biomaterials, nano/micro-patterning and surface modification. He has authored more than 300 publications and holds 18 issued patents. He has given more than 140 plenary and invited lectures at conferences. He is Associate Editor of Journal of Materials Chemistry B; Editorial Board of Journal of Bioactive and Compatible Polymers, Journal of Tissue Engineering and Regenerative Medicine, Regenerative Biomaterials and Biomedical Materials; Advisory Board of Biomaterials Science. He has been selected Fellow of the Royal Society of Chemistry in 2015, Fellow of American Institute for Medical and Biological Engineering in 2017 and Fellow of International Union of Societies for Biomaterials Science and Engineering in 2020.

John Crean

(University College Dublin)

Professor Crean has been engaged in research in the general area of cell-matrix interactions since completing his PhD in 1998 at the University of Bristol, firstly in the biotechnology industry and latterly in an academic setting. He was a recipient of a Dublin Molecular Medicine Fellowship in 2001, enabling a return to academia in the Department of Medicine, University College Dublin. In 2005 he was awarded a prestigious Career Development Award from the Health Research Board and in 2007 was appointed to the Faculty of Science in University College Dublin In 2013 he took up the position of Professor/ Head of Pharmacology. During this time he has been in receipt of >€4,000,000 in funding from agencies including the Irish Health Research Board, Irish Research Council, the Wellcome Trust and Science Foundation Ireland among others. He has established himself as a world-leading expert on CCN proteins and published extensively in this area. Most recently, his group have characterized the complex interaction between CTGF and TGFβ, identifying a miR-302 regulatory loop implcated in the aquisition and maintenence of stemness. Their current studies focus is the exertion of biological and biophysical control of organoid development that define the micro-environmental parameters controlling stem cell differentiation, in parallel with the functional dissection of transcriptional regulatory elements that control cell fate. They have adapted the use of iPSC derived kidney organoids, so called "mini-kidneys", to investigate a novel molecular switch comprising processes that regulate chromatin access during specification and disease. These studies have been published in leading journals including The Journal of Biological Chemistry, The FASEB Journal, The Journal of Immunology, The Biochemical Journal, FEBS,

IOVS and The Journal of Cell Science. Continuing efforts supported by a SFI Investigator award and the SFI Research Centre for Medical devices (CURAM) are focused on developing targeted strategies which can be better facilitated by a complete understanding of the extracellular microenvironment, in particular how this impactes the chromatin landscape of diabetes.

Antonio D'Amore

(Ri.MED Foundation/University of Pittsburgh)

Dr. Antonio D'Amore is a Research Assistant Professor in the Departments of Surgery and Bioengineering at the University of Pittsburgh. Dr. D'Amore also serves as the group leader and head of the cardiac tissue engineering program at Fondazione RiMED. RiMED is an international partnership between the Italian Government, the University of Pittsburgh, and the University of Pittsburgh Medical Center (UPMC), aiming to establish a world-class biomedical research and biotechnology center in Europe. His middle-term mission as a RiMED investigator is to establish a successful cardiovascular tissue engineering program in Italy at the Biomedical Research and Biotechnology Center. The RiMED Cardiac Tissue Engineering laboratory was established in 2020 and is located in Palermo-Italy. Since 2008, Dr. D'Amore has been a bioengineering industry consultant in both the U.S. and Italy. He is the author of more than 170 publications, including peer-reviewed journal articles (>48), book chapters (2), international conference abstracts and extended abstracts (>110), biomedical devices patents applications (11), U.S. patents (4), and software to model biological systems (2). The value of this intellectual property has been recognized by several professional societies, translational science, and career awards such as the National Academy of Inventors (senior member), the "Strazzabosco's" award for young engineers, or the Coulter's Foundation Translational Research award. From 2007, he has been the recipient of 2 pre-doctoral, 2 post-doctoral, and a number of other research awards, he obtained as P.I. or Co-I, which cumulatively secured funding for more than M. Dr. D'Amore is the co-founder and Chief Technology Officer of "Neoolife" a Pitt startup focusing on tissue engineering heart valve technology. Dr. D'Amore's research seeks to couple a mechanistic understanding of the relationship between scaffolds micro-structure, mechanics, and endogenous tissue growth with the development of novel biomaterials for tissue engineering strategies. The focus of his research is upon unmet clinical needs in cardiovascular diseases. Recent areas of interest include: quantitative histology and biomaterials micro-structure image-based analysis, structural modeling strategies to guide tissue engineering scaffold fabrication, mechanical and topological conditioning for tissue elaboration, the development of cardiac restrain devices, vascular grafts, and engineered heart valves. Dr. D'Amore's project funding comes from the National Institutes of Health, European Research Council (ERC-CoG BIOMITRAL with host RiMED Italy), RiMED Foundation, The Wallace H. Coulter Foundation, and the University of Pittsburgh.

Andrew Daly

(Biomedical Engineering, and CÚRAM, at the National University of Ireland, Galway, Ireland)

Dr Daly received a PhD in Biomedical Engineering from Trinity College Dublin in 2018. From 2018 to 2021, he was a Postdoctoral Fellow in Bioengineering at the University of Pennsylvania, and in January 2021, he was appointed as an Assistant Professor in Biomedical Engineering at NUI Galway. His research group at CÚRAM, the Science Foundation Ireland Research Centre for Medical Devices at NUI Galway, focuses on developing new approaches to bioprinting inspired by organ morphogenesis. Recent work has been published in the top journals in the field, including Nature Communications, Nature Reviews Materials, Cell, Biomaterials, Advanced Science, Acta Biomaterialia, Advanced Healthcare Materials, and Biofabrication, with over 1800 citations in the last seven years (h-index 16). The work has been supported by the American Heart Association, Enterprise Ireland, and Science Foundation Ireland.

Patricia Dankers

(Eindhoven University of Technology)

Patricia Y.W. Dankers, PhD, PhD, is professor in Biomedical Materials & Chemistry in the Institute for Complex Molecular Systems (ICMS), and the department of Biomedical Engineering at the Eindhoven University of Technology (TU/e).
Her particular research interests are on the design and synthesis of functional biomaterials. In her research, the chemistry of different supramolecular polymeric biomaterials held together via directed, non-covalent interactions is the central theme. Besides obtaining fundamental knowledge on the functioning of supramolecular biomaterials in a biological environment, translation of these biomaterials to biomedical applications is an important task within her research group.
She studied chemistry at the Radboud University of Nijmegen, the Netherlands, where she majored in biochemistry and organic chemistry. During her PhD in natural sciences and chemistry at the Eindhoven University of Technology in the group of prof.dr. E.W. (Bert) Meijer, she combined her fascination for biochemistry and supramolecular chemistry. She developed and studied supramolecular bioactive biomaterials by introducing a modular approach. After her PhD defense in 2006, she worked for the company SupraPolix in Eindhoven, and in the laboratory of Pathology and Medical Biology at the University Medical Center Groningen where together with prof.dr. Marja J.A. van Luyn she initiated the bioartificial kidney project in the Netherlands. She defended her second PhD thesis in medical sciences on kidney regenerative medicine in 2013. In 2010 she worked in the Institute for BioNanotechnology in Medicine at the Northwestern University in Chicago, USA, in the research group of prof.dr. Samuel I. Stupp. She started as an assistant professor in 2008, became an associate professor in 2014, and is full professor since 2017 at the Eindhoven University of Technology.
She is a NWO Veni and Vidi laureate (2008, 2017) and received an ERC starting (2012) and ERC Proof of Concept grant (2017). She has been awarded various grants and awards, such as the Journal of Polymer Science Innovation Award at the American Chemical Society national meeting (2019), the KNCV Gold Medal (2020), and the Ammodo Award for Fundamental Science (2021). From 2011-2013 she has been a member of the first Young Dutch Health Council, and from 2015-2020 a member and board member of the Young Academy of the Royal Dutch Society of Arts and Sciences (KNAW). She has been the chairman of the Netherlands Society of Biomaterials

(NBTE) for 4 years, and is currently the chairman of the Round Table Chemistry of the Dutch Science Foundation (NWO). Furthermore, she founded the Eindhoven Young Academy of Engineering in 2017. She considers the promulgation of science to society a very important topic. Therefore, she developed and initiated a teaching program at primary schools about performing research. Furthermore, she is involved in setting up companies in biomaterials development, and she values patenting of important findings in order to translate research to the clinic. She is a co-founder of the spin-off company UPyTher, that deals with intraperitoneal drug delivery solutions (2020).

Daniela Duarte Campos

(Heidelberg University)

Family name, First name: Duarte Campos, Daniela
Date of birth: 02.07.1986
Nationality: Portuguese

EDUCATION

2019 Master of Science, Laboratory Animal Science
International Academy, RWTH Aachen, Germany
2016 PhD, Biomaterials
Dept. of Biomaterials Research, RWTH Aachen University Hospital, Germany
2010 Master of Science, Biomedical Engineering
Department of Bioengineering, University of Minho, Portugal

CURRENT POSITIONS

2021 Tenure-track Professor of Bioengineering
Centre for Molecular Biology (ZMBH), Heidelberg University, Germany
2021 Principal Investigator
Cluster of Excellence 3DMM2O, Heidelberg University, Germany

PREVIOUS POSITIONS

2020 – 2021 Principal Investigator
De Laporte Lab, AME Institute, RWTH Aachen University Hospital, Germany
2019 – 2020 Visiting Postdoc
Heilshorn Lab, Dept. of Materials Science & Engineering, Stanford University, USA
2016 – 2019 Leader of the Bioprinting Lab
Dept. of Biomaterials Research, RWTH Aachen University Hospital, Germany
2010 – 2016 PhD candidate
Dept. of Biomaterials Research, RWTH Aachen University Hospital, Germany

FELLOWSHIPS AND AWARDS

2020 Outstanding Reviewer, Journal Biofabrication
2020 Theodore von Karman Fellowship, RWTH Aachen
2019 Postdoc Fellowship to visit Stanford University, German Research Foundation
2019 FELASA A, B and D specialist, RWTH Aachen University Hospital
2019 Best Podium Presentation (3rd place), TERMIS
2018 Julia Polak European Doctorate Award, European Society for Biomaterials
2018 Best Young Investigator Poster Award (3rd place), TERMIS
2017 Reviewer of the Year, Journal Biofabrication
2017 Borchers badge for Doctoral students with distinction, RWTH Aachen
2016 Master Study Fellowship for Laboratory Animal Science (Executive), RWTH Aachen
2016 Best PhD Dissertation Prize (1st place), German Society for Biomaterials

Miroslawa El Fray

(West Pomeranian University of Technology)

Prof. Miroslawa El Fray is Full Professor at the West Pomeranian University of Technology in Szczecin (Poland). She is also Director of the Nanotechnology Centre for Education and Research, and Head of the Department of Polymer and Biomaterials Science. She was a post-doc at the Technical University Hamburg-Harburg, and scientific researcher at the University Bayreuth, Germany. She received a Royal Society fellowship in 2005 at the Imperial College London, UK. She is also recipient of Fulbright STEM Impact Award 2019 from Polish-U.S. Fulbright Commission at The Ohio State University, Oh, USA. She completed 12 projects financed from national and international research funding bodies (NCN, NCBiR, 7FP) and a number of projects in cooperation with DePuy Johnson & Johnson (UK), Uniqema/Croda, Philips (The Netherlands), Honda Europe (Germany). She is already a coordinator of GREEN-MAP project financed from H2020-MSCA-RISE program. She holds 9 patents, including 2 granted by USPTO. She co-authored over 140 publications in JCR indexed journals. She supervised 10 PhDs, 54 master and 24 diploma (engineering) students. Prof. El Fray is Member of International Advisory Board of The Institute of Experimental Medicine of the Czech Academy of Science in Prague, Czech Republic. She is also a CEO of a spin-off company PolTiss sp z o.o. commercializing new injectable photocurable polymers for innovative hernia treatment. Her scientific background spans synthesis, characterization, biodegradation and polymer modification towards specific biomedical applications. She has made extensive contribution to the development of fatty acid-based polyesters, structure-properties relationship of various copolymers and their derivatives, including nanocomposites, chitosan, and photocurable networks.

Giancarlo Forte

(St. Anne's University Hospital)

Giancarlo Forte is Deputy Director for Science of the International Clinical Research Center, an Institute of excellence funded by European Union within St. Anne's University Hospital in Brno, Czech Republic (FNUSA-ICRC).
He serves as Head of the Center for Translational Medicine (CTM) at FNUSA-ICRC and holds an Adjunct Professor position in Cell Biology and Biomaterials at the University of Turku (Finland).
Dr. Forte obtained his Master Degree in Cellular and Molecular Biology in 2000 at the University of Rome Tor Vergata (Italy), where he also completed his PhD in 2005 in Experimental Pathophysiology. After his graduation, he was a visiting researcher at the University Karls Eberhard in Tuebingen, Germany.
Next, he became a postdoctoral fellow at the Italian Institute for Cardiovascular Research (INRC), where he was awarded the Young Investigator Award for his research on cell-matrix interaction in 2008.
During his postdoctoral experience, he was a visiting scientist at Tokyo Women's Medical University (2006), and a visiting researcher at National Institute for Materials Science (NIMS, 2010) in Japan.
In 2010 he was appointed a Senior Researcher position at NIMS, in Japan and MANA Scientist position at the World Premiere Institute for Materials Nanoarchitectonics (MANA) in the same Country.
In May 2013 he accepted a Senior Scientist position in the newly established International Clinical

Research Center (ICRC) of St. Anne's University Hospital, Brno (Czech Republic), where he joined the Integrated Center for Cell Therapy and Regenerative Medicine (ICCT) in 2014. ICCT later became the Center for Translational Medicine (CTM).
CTM laboratories currently host 5 independent research groups (~45 members) from 11 different nationalities. Thanks to the support of european grants, he contributed to found the Competence Center for Mechanobiology in Regenerative Medicine, an Austria-Czech Republic joint initiative and the first research center of this kind in Czech Republic.
His research group focuses mainly on the cell-specific response to pathological extracellular matrix (ECM) remodelling, with special attention to the defects in the regulation of mechanosensitive intracellular pathways as determinants of diseases.
Dr. Forte serves as ad hoc reviewer for a number of international peer-reviewed journals which include Cardiovascular Research, Cell Reports, Journal of Cell Biology, Biomaterials, Nature Materials, Nature Communications.
He is a long term member of Tissue Engineering and Regenerative Medicine International Society (TERMIS), International Society for Stem Cell Research (ISSCR), Stem Cell Research Italy (SCR Italy), the Platform for Advanced Cellular Therapies (PACT), and the European Association for Cardio-Thoracic Surgery (EACTS).

Bernd Giebel

(Institute for Transfusion Medicine; University Hospital Essen)

Bernd Giebel studied biology in Cologne and received his PhD in 1996. 1999 he moved to the Heinrich-Heine-University of Düsseldorf, to work with human hematopoietic stem and progenitor cells. In 2008 he switched to the University Hospital Essen, continued his studies on human somatic stem cells and started to work with EVs in 2009. Setting a focus on mesenchymal stem/stromal cell-derived EVs (MSC-EVs), together with collaboration partners his group demonstrated the therapeutic potential of MSC-EVs in a human GvHD-patient and in different animal models. It is his goal to efficiently translate MSC-EVs into the clinics and to set up appropriate quality control platforms. BG is the president of the German Society of Extracellular Vesicles (GSEV), co-chairing the exosome working group of the International Society of Gene and Cell Therapy (ISCT) and part of the scientific advisory board of two SME companies, Innovex Therapeutics and Mursla LTD. Furthermore, he is a consultant of FUJIFILM Wako Chemicals Europe GmbH and founding director of Exosla LTD.

Thomas Groth

(Department Biomedical Materials, Martin Luther University Halle-Wittenberg)

Thomas Groth is full Professor of Biomedical Materials at Martin Luther University Halle-Wittenberg, Germany. He worked on development of membranes for artificial and bioartificial organs and contributed to the understanding of blood and tissue compatibility of biomaterials. His current activities focus on biomimetic surface modification of implant materials and development of hydrogels for engineering skeletal tissues and control of inflammatory response. He graduated in biology (Diploma) and obtained his PhD in biophysics at Humboldt University Berlin, Germany. He did his postdoctoral thesis (DSc) at University of Potsdam in the area of biomaterials. Thomas Groth coordinated several EU funded and national projects. He was President of European Society for Artificial Organs, is

currently Speaker of International PhD School AGRIPOLY and Board Member of Interdisciplinary Center of Materials Science at Martin Luther University. In addition, he serves the International Federation for Artificial Organs as Secretary Treasurer and is cooopated Board Member of the European Society for Artificial Organs. Thomas Groth is also European Editor of the journal Artificial Organs and Editorial Board Member in several other journals with focus on biomaterials science/engineering and tissue engineering. He published more than 200 papers in international journals and books. His current h-index is 46 (Google Scholar).

Debbie Guest

(Royal Veterinary College)

Debbie completed a BSc in genetics at the University of Leeds in 2002 and then went on to undertake a PhD on the transcriptional control of neuronal differentiation in stem cells, which she completed in 2005. Following her PhD she undertook a post-doctoral position at the Equine Fertility Unit in Newmarket to derive and characterise horse embryonic stem cells. In 2007 she moved to the Animal Health Trust and started to investigate the therapeutic potential of embryonic stem cells for treating horse tendon injuries. Since then she has expanded her research into the derivation of induced pluripotent stem cells from horses, dogs and cats with a focus on both their therapeutic applications and use in disease modelling. Debbie joined the RVC as a senior research fellow in 2020.
Debbie is a member of the International Society for Stem Cell Research (ISSCR) and the Tissue Engineering and Regenerative Medicine International Society (TERMIS). She is a reviewer for numerous scientific journal and grant funding bodies and is an associate editor for Frontiers in Veterinary Science, Regenerative Medicine.

Greg Hudalla

(University of Florida)

Dr. Hudalla received a B.S. in Chemical Engineering from the Illinois Institute of Technology in 2004, a M.S. in Biomedical Engineering from the University of Wisconsin in 2006, and a Ph.D. in Biomedical Engineering from the University of Wisconsin in 2010. Dr. Hudalla was a post-doctoral fellow at the University of Chicago and Northwestern University from 2010-2013 through support from an NIH National Research Service Award. Dr. Hudalla is currently an Associate Professor in the J. Crayton Pruitt Family Department of Biomedical Engineering at the University of Florida, where he has been since 2013. Dr. Hudalla's research program seeks to understand and employ the physical phenomenon of self-assembly as a means to understand and manipulate galectin-glycan interactions, as well as to create new vehicles for biotherapeutic drug delivery. Dr. Hudalla has authored more than 45 publications, is co-editor of the book "Mimicking the Extracellular Matrix: The Intersection of Matrix Biology and Biomaterials", and holds 3 US patents, with another 10 currently pending. Dr. Hudalla has received the Cellular and Molecular Bioengineering Young Innovator award, the Journal of Materials Chemistry B, Biomaterials Science and Molecular Systems Design & Engineering Emerging Investigator awards, a National Science Foundation RAISE award, the National Institute of Biomedical Imaging and Bioengineering Trailblazer award, the National Science Foundation CAREER award, the University of Wisconsin Alumni Early Career Achievement award, the Cade Prize for Invention and Creativity, the National Institute of General Medical Sciences Maximizing

Investigators' Research Award, a Pruitt Family Endowed Faculty fellowship from the UF Department of Biomedical Engineering, and was named a UF Term Professor from 2018-2021.

Gunil Im

(Dongguk University BMC)

Gun-il Im, M.D., Ph.D is the President of International Combined Orthopaedic Research Societies (ICORS), the President-Elect of Osteoarthritis Research Society International (OARSI), and also the Past President of Korean Society for Biomaterials (KSBM), and the Past President of Korean Society for Cartilage and Osteoarthritis.
Dr. Im is currently Professor in Department of Orthopaedics, Dongguk University Ilsan Hospital, Goyang, Korea. He is also leading the Integrative Research Institute for Regenerative Biomedical Engineering (IRBE) of Dongguk University, a specialized center dedicated to stem cell and tissue regeneration research.
Dr. Im received his M.D in Seoul National University in 1988 and finished his training as an orthopaedic surgeon at Seoul National University Hospital in 1993. He then obtained his Ph.D. degree in Korea University in 1999. Dr. Im began his academic career as an Assistant Professor at Dept. of Orthopaedics, Hallym University Hospital in 1996 and moved to Dept. of Orthopaedics, Dongguk University Ilsan Hospital in 2006 and has been working in the institution until now. Dr. Im has participated in global academic activity in the field of musculoskeletal research during last two decades and possesses numerous connections with researchers overseas.
Dr. Im has been working on the field of stem cell and tissue regeneration of musculoskeletal system since 1996. He has published 160 peer-reviewed scientific papers in international journals and holds 18 national and international patents. He has accumulated rich knowledge in the research on the regeneration of bone and cartilage regeneration. Dr. Im has been performing multidisciplinary research in cooperation with scientist and engineers of various fields to translate the research product into clinical application. Based on his works, he has been awarded several prestigious awards from the Korean governments, academic societies and his university.

Keita Ito

(Eindhoven University of Technology)

Prof.Dr. Keita Ito received his doctorate in Medical Engineering and Medical Physics from the Massachusetts Institute of Technology and his medical degree from Harvard Medical School. Currently, he is Vice Dean and full professor in the Dept. of Biomedical Engineering at the Eindhoven University of Technology, where he leads the Orthopaedic Biomechanics group. This group combines numerical/experimental and engineering/biological methods to elucidate degenerative processes in bone, cartilage, disc and tendons/ligaments, as well as regenerative strategies thereof. He also is a professor in the Dept. of Orthopaedics at the University Medical Center Utrecht where he works on the mechanobiology of musculoskeletal regenerative medicine. He has co-authored over 200 peer-reviewed publications and is on the editorial board of Biomech Model Mechanobiol and is a deputy-editor of the Global Spine Journal. Recently, he was awarded an ERC-AdG to investigate the etiology of adolescent idiopathic scoliosis.

Diana Jurk

(Mayo Clinic)

Dr Diana Jurk is originally from Germany where she graduated with a degree in Natural Sciences from the University of Freiberg in 2004. Following her degree, she conducted biomedical research at Bayer (one of the largest German multinational pharmaceutical companies), first in Wuppertal and then in Leverkusen (where she was awarded a Master's degree).
Having decided to move to academia, Dr. Jurk was first a research assistant at the Uniklinik in Freiburg, Germany (2005-2007) and then conducted her PhD studies at Newcastle University in the UK (2007-2012), in the area of liver senescence and inflammation. Following her PhD, Dr. Jurk did a post-doc in the lab of Prof. Thomas von Zglinicki at Newcastle University on the subject of senescence and neurodegeneration.
In 2015, Dr. Jurk was awarded the Newcastle University Faculty fellowship and in 2018 the prestigious springboard award from The Academy of Medical Sciences which allowed her to direct an independent research program. In 2018 she moved her research team to Mayo Clinic, Rochester and was appointed Assistant Professor of Physiology. In 2021 she was promoted to Associate Professor of Physiology.
Dr. Jurk's work has led to new insights into the mechanisms driving the process of cellular senescence in the context of liver disease and neurodegeneration. Her work, published in Nature Communications, has demonstrated a key role for senescence in Non-alcoholic fatty liver disease. More recently, her team published in Cell Metabolism and Aging Cell the first evidence for the involvement of senescence in neuropsychiatric diseases and during normal aging.

Ryuji Kato

(Nagoya University)

Dr. Kato is a Principal Investigator of Cell & Molecular Bioengineering Laboratory in Graduate School of Pharmaceutical Sciences, Nagoya University since 2012. He has graduated from engineering faculty in Tohoku University, and earned bioscience master degree in Nara Institute of Science and Technology (NAIST). He has earned Ph.D. in Bioinformatics/Biotechnology from Graduate School of Engineering, Nagoya University in 2004. He is also a founder and CEO of his start-up Quastella Inc.
He has published more than 90 papers, 21 patents in the field of medical engineering. He has made more than 90 industrial collaborations and contributed to producing 12 commercial products in the field of cell culture engineering, such as automatic cell culture systems, cell image analysis software, culture vessel, and culture medium. Further, he has been consulting the major cell manufacturing companies in Japan as a leading cell engineering researcher bridging academia and industry.

Daniel Kelly

(Trinity College Dublin)

Prof Daniel Kelly leads a multidisciplinary musculoskeletal tissue engineering group based in the Trinity Centre for Biomedical Engineering. The goal of his lab is to understand how environmental factors regulate the fate of adult progenitor cells and the tissues they produce. This research underpins a more translational programme aimed at developing novel tissue engineering and 3D bioprinting strategies to regenerate damaged and diseased musculoskeletal tissues. To date he has published over 200 articles in peer-reviewed journals. He is the recipient of four European Research Council awards (Starter grant 2010; Consolidator grant 2015; Proof of Concept grant 2017; Advanced grant 2021).

Niels B. Larsen

(Technical University of Denmark)

Niels B. Larsen received his PhD in surface chemistry from University of Copenhagen in 1997, and has been a professor since 2003, first at Riso National Laboratory in Denmark and since 2007 at Technical University of Denmark. His current research interests focus on technologies to recapitulate the function of the human vascular network for tissue engineering applications, mainly targeting advanced in vitro 3D tissue culture but also with a perspective towards extracorporeal and implantable devices. The key technology platform is high-resolution stereolithographic 3D printing in diffusion-open materials for which he has developed custom-build multi-color stereolithography systems and matching printing resins, which enable spatially selective solidification of different resin components to produce monolithic devices with locally widely varying diffusive and mechanical properties.

Christine Le Maitre

(Sheffield Hallam University)

Christine is Head of the Tissue Engineering and Biomechanics research group in the Biomolecular Sciences Research Centre at Sheffield Hallam University. Her main research focus is investigating the cellular pathogenesis of musculoskeletal conditions, in particularly intervertebral disc degeneration and its links to low back pain, osteoarthritis and bone physiology, working in close collaboration with relevant clinical partners. With a particular interest in the interaction of cells, biomechanics and matrix biology. Utilising this knowledge to develop regenerative strategies for repair and regeneration. Christine has developed a number of innovative approaches combining biomaterials and regenerative cell sources to promote the regeneration of the intervertebral disc, with a particular emphasis of targeting the pathophysiology of disc degeneration. Christine is an internationally renowned scientist with over 22 million in grant income, 2 patent families and >90 publications to date with expertise in several multidisciplinary fields, a current H index of 40 and i10 index 69, with >7,600 citations on her publications to date. She has been invited to present her research at a number of national and international meetings, she is past chair of ORS Spine section and an elected committee member of Society of Back Pain Research and is an AO UK delegate and DISCs Chairlady. She has supervised 15 PhD students to completion and currently supervises 11 further students and is

the Head of Research Degrees for the Institute of Industry and Innovation at Sheffield Hallam University.

Catherine Le Visage

(Inserm Regenerative Medicine and Skeleton)

Catherine Le Visage is a Research Director and the Deputy Director of the Regenerative Medicine and Skeleton (RMeS) laboratory at the University of Nantes, France. She was trained as a Pharmacist, received her PhD in Pharmaceutical Technologies in Paris then performed a post-doctoral training in the BME Department of the Johns Hopkins University (Baltimore, USA) in Prof. K. Leong's laboratory with a focus on biomaterials for regenerative approaches. In 2007, she joined with a tenured position the French National Institute of Health and Medical Research (INSERM) to investigate chemically cross-linked polysaccharide hydrogels.
As a group leader, her most recent works have focused on innovative hydrogels as i) carriers of cells or bioactive molecules in the context of IVD disease and osteoarthritis and ii) tools for stem cell-based organogenesis.
She is an elected member of the TERMIS-EU Council (2019-2025), and has been appointed Secretary of the Executive Committee. She is a member (2020-2022) of the Editorial Advisory Board of "ACS Applied Materials & Interfaces", and a reviewer for national and international funding agencies (Canada First Research Excellence Fund, European Science Fondation, L'Oreal/UNESCO).
She has coauthored 73 publications (h-index 32) and 11 patents, and has given 60 invited lectures/seminars at national and international conferences. Web of Science ResearcherID: E-5460-2011

Riccardo Levato

(Utrecht University)

Riccardo Levato is Associate Professor of Translational Bioengineering and Biomaterials at the Department of Clinical Sciences (Faculty of Veterinary Medicine, Utrecht University), and a Principal Investigator both at the Regenerative Medicine Center Utrecht and at the Department of Orthopedics of the University Medical Center Utrecht. His research interests focus on the development of novel biofabrication strategies and cell-instructive biomaterials to create bioprinted, lab-made tissue models and transplantable engineered grafts. Integrating expertise in engineering, materials science and stem cell biology, these efforts aim both to understand and mimic the multifaceted architectural and biochemical structure of living tissues in order to develop effective treatments for human and veterinary healthcare. In 2020 he was awarded a Starting grant from the European Research Council on the development of a novel volumetric bioprinting technology for organoid research and to engineer functional bone marrow analogues in vitro. In addition, since 2021, he is coordinator of a European consortium (ENLIGHT), funded under the Future and Emerging Technologies scheme (European Innovation Council pilot), aiming at developing biofabricated pancreas units to study treatments for diabetes. To date, he has published 52 peer-reviewed articles international journals, co-authored 2 book chapters and 2 patent applications, and he secured > 13 million euros in research funds for his group and related consortia. In total, he has been (co-)supervisor of 16

PhD students (4 completed, 9 ongoing). For his work, he was conferred several awards including a Orthoregeneration Network Fellowship by the International Cartilage Repair Society (ICRS), the 2016 Wake Forest Institute for Regenerative Medicine Young Investigator Award, multiple presentation and travel awards, and the 2021 Jean Leray award from the European Society for Biomaterials. Riccardo is also serving on the Board of Directors of the International Society for Biofabrication. Prior to moving to Utrecht, he worked in several research groups working in the fields of Biomaterials, Regenerative Medicine and Biofabrication: 3Bs, University of Minho (Portugal); BioMatLab, Technical University of Milan (Italy), the Biomaterials for Regenerative Therapies group at the Institute for Bioengineering of Catalonia (IBEC, Spain), and he holds a cum laude PhD in Biomedical Engineering from IBEC and from the Technical University of Catalonia (Barcelona, Spain).

Malgorzata Lewandowska-Szumiel

(Medical University of Warsaw)

Prof. Malgorzata Lewandowska-Szumiel, PhD, FBSE, holds joint appoints as a full professor at the Department of Histology and Embryology of the Medical University of Warsaw. She is also the Founding Director of the Laboratory for Cell Research and Application (LBBK) – the Medical University of Warsaw Unit, which holds GMP certificate and authorization to produce cell-containing products for clinical trials. LBBK also serves as a Cell and Tissue Bank as well as a Biobank – member of a Polish Biobanking Network.
Prof. Malgorzata Lewandowska-Szumiel was graduated from the Warsaw University of Technology in Materials Science and Engineering (M.Sc., Eng.), while she completed her PhD and habilitation degrees in Medical Sciences and holds a Titular Professorship in Medicine. Such combination makes her a multi-disciplinary translational scientist. She is directing many interdisciplinary projects which involve materials scientists, biologists and clinicians. She published numerous scientific articles and invented several patents in tissue engineering. She served as an expert for the EU negotiations on the Regulation on ATMPs.
She holds the honorary status of "Fellow, Biomaterials Science and Engineering" (FBSE) from International Union of Societies of Biomaterials Science and Engineering and was awarded by the Polish Academy of Sciences for a series of works on the interaction of cells with implantable materials.

Silvia Lopa

(Cell and Tissue Engineering Laboratory, IRCCS Galeazzi Orthopaedic Institute)

I work as a PostDoctoral Researcher at the Cell and Tissue Engineering Laboratory of the IRCCS Galeazzi Orthopaedic Institute (Milan, Italy). My research activity primarily aims at the development of advanced microscale models, with a specific focus on joint tissues and osteoarthritis. In the latest years, I have led as Principal Investigator a project funded by the Italian MoH aimed at generating a microfluidic model of the osteoarthritic joint to investigate monocyte extravasation and screen anti-chemokine therapies.
Currently, I am focusing on the development of several microfluidic models including multiple joint elements to investigate the crosstalk of tissue-specific cells and screen novel therapies for osteoarthritis. My research activity is also focused on the role of macrophages in osteoarthritis-related inflammation and on the possibility to modulate macrophage phenotype to achieve a

healing-friendly environment.
Since the beginning of my research activity, I have been involved in many TERMIS initiatives. During my PhD, I participated to the TERMIS Summer Schools that have been held in Sheffield and Riva del Garda. In 2017, I was elected as a SYIS member of the Strategic Alliance Committee, which promotes the interaction between TERMIS and other Scientific Societies.

Jos Malda

(UMC Utrecht)

Professor Jos Malda is Head of Research at the Department of Orthopaedics, University Medical Center Utrecht and the Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Utrecht. He also leads the Utrecht Biofabrication Facility. He received his MSc degree in Bioprocess Engineering (Wageningen University, 1999) and completed his PhD on Cartilage Tissue Engineering in 2003 (University of Twente). He subsequently accepted a research fellowship at the Institute of Health and Biomedical Innovation, (Queensland University of Technology, Brisbane, Australia). In 2007, Dr. Malda was awarded a fellowship that allowed him to establish his research group in Utrecht. Dr. Malda has published over 175 articles in peer-reviewed international journals, attracted over 20 million Euro in research funding and holds an ERC Consolidator grant. Further, he is one of the initiators of the first international master's program in Biofabrication. From 2014-2018, he was the president of the International Society for Biofabrication (ISBF) and currently is Secretary General of the International Cartilage Regeneration and Joint preservation Society (ICRS). He is a pioneer in the field of biofabrication and his research aims to promote tissue regeneration by recreating 3D biological environments with the aid of biofabrication technologies. Bioprinted constructs are evaluated in ex vivo defect models and bioreactors to provide tissue maturation and validation as close as possible as that occurring in the in vivo situation. Moreover, promising strategies are translated towards in vivo models with potential impact in human and veterinary medicine.

João Mano

(University of Aveiro)

João F. Mano is a Full Professor at the Chemistry Department of University of Aveiro, Portugal, and vice-director of the Associate Laboratory CICECO – Aveiro Instituto of Materials, where he is directing the COMPASS Research Group. His research interests include the use of advanced biomaterials and cells towards the progress of multidisciplinary concepts to be employed in regenerative and personalised medicine. In particular, he has been applying biomimetic and nano/micro-technology approaches to polymer-based biomaterials and surfaces in order to develop biomedical devices with improved structural and (multi-) functional properties, or in the engineering of microenvironments to control cell behaviour and organization, to be exploited clinically in advanced therapies or in drug screening. He is the Editor-in-Chief of Materials Today Bio (Elsevier). He has been coordinating or involved in many national and European research projects, including 2 Advanced Grants and 3 Proof-of-Concept Grants from the European Research Council. João F. Mano has received different honours and awards, including two honoris causa doctorates (Univ. of Lorraine and Univ. Utrecht) and was elected fellow of the European Academy of Sciences (FEurASc), Biomaterials Science & Engineering (FBSE) and American Institute of Medical and Biological Engineering (FAIMBE).

Matteo Moretti

(Ente Ospedaliero Cantonale, Regenerative Medicine Technologies Lab)

Matteo Moretti is the director of the Regenerative Medicine Technologies Laboratory at the Ente Ospedaliero Cantonale (EOC), Adjunct Professor at the Biomedical Sciences Faculty of Università della Svizzera Italiana (USI) Lugano, Switzerland and director of the Cell and Tissue Engineering Laboratory at the IRCCS Galeazzi Orthopedic Institute, Milan, Italy. He previously worked as a post-doc fellow in the Langer Lab at the Massachusetts Institute of Technology, Harvard-MIT's Division of Health Science and Technology. Both of his titles, B.Eng (Polytechnic of Milan) and M.Sc (Trinity College Dublin, Ireland) are in Bioengineering. He obtained a European doctorate in 2005 in Bioengineering at Polytechnic of Milan, sharing his research period with the Tissue Engineering Laboratory of Prof. I. Martin at University of Basel. His main research interests are advanced cell culture technologies for musculoskeletal tissues and their pathologies. In particular, he focused on engineered tissues and in vitro models, 3D tumor and fibrosis models, tissue vasculature and multi-scale bioreactor systems as key tools for viable and accessible cellular and tissue therapies. His scientific awards include a N.A.S.A. Tech Brief Award, for the development of scientific or technical innovations. Industrially, he has been coordinator of European projects for Fidia Advanced Biopolymers, has a licensed patent and has been co-founder of 2 biotech start-ups (SKE S.R.L. and Cellec A.G.) focused on bioreactor technologies. He has been member of TERMIS since its foundation, co-founder of SYIS section and TERMIS-EU Treasurer and Council Executive Committee member (2015-19). He authored more than 100 publications in international peer-reviewed scientific journals.

Lorenzo Moroni

(Maastricht University - MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration department)

Prof. Dr. Lorenzo Moroni received his Ph.D. cum laude in 2006 at University of Twente on 3D scaffolds for osteochondral regeneration, for which he was awarded the European doctorate award in Biomaterials and Tissue Engineering from the European Society of Biomaterials (ESB).
Since 2014 he works at Maastricht University, where he is a founding member of the MERLN Institute for Technology-Inspired Regenerative Medicine. In 2016, he became full professor in biofabrication for regenerative medicine. Since 2019, he is chair of the Complex Tissue Regeneration department and since 2022 director of MERLN.
In 2014, he received the Jean Leray award from the ESB and an ERC starting grant. In 2016, he also received the Robert Brown Award from TERMIS. In 2017, he was elected as faculty of the Young Academy of Europe and in the top 100 Italian scientists within 40 worldwide by the European Institute of Italian Culture.
His research group interests aim at developing biofabrication technologies to generate libraries of 3D scaffolds able to control cell fate, with applications spanning from skeletal to vascular, neural, and organ regeneration. From his research efforts, 3 products have already reached the market.

Francesco Moscato

(Center for Medical Physics and Biomedical Engineering, Medical University of Vienna)

Francesco Moscato received the PhD in Industrial Bioengineering from the University of Calabria (Italy) in 2008. He was Visiting Scholar at Columbia University (New York USA) in 2014. Since 2015 he is Associate Professor at the Medical University of Vienna.
The research of Francesco Moscato focuses on two main areas: Medical Additive Manufacturing: investigation of how 3d-printing can improve surgical and interventional procedures, medical device prototyping, tissue engineering and medical education. Cardiovascular Bioengineering: research and development of methods and devices improve diagnostics and provide support to a range of cardiovascular pathologies.
He is author of 65 original articles and more than 30 invited talks (twice at a Gordon Research Conference). He was Secretary General (2013-17) and President (2018-19) of the International Society for Mechanical Circulatory Support. Francesco Moscato has been Principal Investigator/ Site Coordinator in 8 international and national research grants (for a cumulative funding of about 4.2 Mio EUR).

Nuno Neves

(3B's Research Group / University of Minho)

Nuno M. Neves is an Associate Professor with Habilitation at the 3B's Research Group of the University of Minho, Portugal. He has been involved in biomaterials research since 2002 and is currently President of the I3Bs Research Institute. His main area of research is focused on tissue engineering and regenerative medicine strategies using stem cells and biodegradable biomaterials for advanced drug delivery systems, scaffolds and medical devices. He is the author of 219 publications with an h-factor of 46 and is the co-inventor of 7 patents.
He currently serves as Editorial Board member of Biomolecules, Associate Editor in Tissue Engineering and Regenerative Medicine (specialty section of Frontiers in Bioengineering and Biotechnology) and the Elsevier journal Regenerative Therapy.
He is routinely invited to review research grants and research proposals for the European Commission and for various funding agencies in Portugal, Argentina, Austria, Czech, France, Georgia, Germany, Netherlands, New Zealand, Singapore, Slovakia, Slovenia, UK and USA and advisory panels in France and Croatia.
He is an elected member of the Board of Governors of the European Society for Artificial Organs (ESAO) and is currently the responsible for the Tissue Engineering Working Group of the ESAO.

Tomasz Nowakowski

(UCSF)

I received my PhD from the University of Edinburgh in 2012 and completed my postdoctoral training in 2017 with Arnold Kriegstein. I am an Assistant Professor at the University of California San Francisco, where my lab focuses on uncovering the developmental origins of cell type heterogeneity in the human nervous system. In particular, I employ systems biology techniques to study the molecular mechanisms that regulate fate competence of neural stem

cells, and regulate the assembly of neural circuits. We currently lack scalable technologies to address these questions in the human and non-human primate nervous system, and towards being able to address the long-standing questions about contribution of specific cell types to brain development and brain function.

Fergal O'Brien

(RCSI)

Prof. Fergal O'Brien is a leading innovator in the development of advanced biomaterials for regenerative medicine. Since his faculty appointment, he has published over 250 journal articles, supervised over 45 doctoral students to completion, filed over 20 patents/disclosures and translated a number regenerative technologies for bone and cartilage repair to the clinic through spin-out formation and licensing to industry. He has presented over 100 invited talks and has a current h-index of 81 (Feb 2022). He is a recipient of three prestigious European Research Council Awards including an ongoing €3 million ERC Advanced Grant. Other accolades include a Fulbright Scholarship (2001), New Investigator Recognition Award by the Orthopaedic Research Society (2002), Science Foundation Ireland, President of Ireland Young Researcher Award (€1.1. million, 2004), Engineers Ireland Chartered Engineer of the Year (2005), Anatomical Society New Fellow of the Year (2014), Silver Medal from the Bioengineering Section of the Royal Academy of Medicine in Ireland, Fellowship of Engineers Ireland (2013) and the European Alliance for Medical & Biological Engineering Science (2016). In 2018, he was elected as a member of the Royal Irish Academy, the highest academic honour in Ireland and was the recipient of the Science Foundation Ireland Industry Partnership Award in 2020 and Knowledge Transfer Ireland Commercialisation Impact award in 2021.

Mikolaj Ogrodnik

(Ludwig Boltzmann Research Group SHoW - Senescence and Healing of Wounds)

Mikolaj Ogrodnik is a group leader at the Ludwig Boltzmann Research Group Senescence and Healing of Wounds (LBG-SHoW). His research interests focus on wound healing and the biology of cellular senescence in vivo and the impact of senescent cells on a variety of age-related conditions and disorders such as wound healing, liver steatosis, memory impairment, osteoarthritis, diabetes, atherosclerosis, cardiac dysfunction, skin aging, and skin chronic pathology. Dr. Ogrodnik is an expert in the field of cellular senescence, tissue regeneration, and animal models of wound healing and senescence induction. He has authored 25 publications, with 9 as first/senior author, and currently has an h-index of 19 (Scopus) with over 4000 citations (Google Scholar).

Aleksandr Ovsianikov

(Research Group 3D Printing and Biofabrication, TU Wien)

Prof. Ovsianikov is head of the group “3D Printing and Biofabrication” at the TU Wien (Vienna, Austria). His research is dealing with the use of additive manufacturing technologies and bioprinting for tissue engineering and regeneration. Prof. Ovsianikov was awarded prestigious ERC Starting Grant in 2012 and an ERC Consolidator Grant in 2017 for projects aimed at these topics. He is an elected board member of the International Society for Biofabrication (biofabricationsociety.org). Together with Prof. Mironov and Prof. Yoo he is an editor of a living book project “3D Printing and Biofabrication” published by Springer in cooperation with Tissue Engineering and Regenerative Medicine International Society (TERMIS). Prof. Ovsianikov also a co-founder and a head of research of a TU Wien spin-off UpNano GbmH (upnano.at), aimed a commercialization of high-resolution 3D printing and Bioprinting, which received multiple awards, including the Austrian Startup of the year 2019.

Sean Palecek

(University of Wisconsin - Madison)

Sean Palecek is the Milton J. and A. Maude Shoemaker Professor and Vilas Distinguished Achievement Professor in the Department of Chemical & Biological Engineering at the University of Wisconsin – Madison. Sean is also the Director for Research for the National Science Foundation Center for Cell Manufacturing Technologies (CMaT) and the Associate Director for Research Innovation for the Forward BIO Institute. Sean is also a Fellow at the Allen Institute for Cell Science (Seattle, WA). Sean’s research lab studies how human pluripotent stem cells (hPSCs) sense and respond to microenvironmental cues in making fate choices. Sean’s lab has generated novel mechanistic insight and developed protocols for differentiation of hPSCs to cardiovascular and neurovascular cell types. They strive to engineer fully-defined, animal component-free differentiation platforms, compatible with biomanufacturing of cells and tissues for in vitro and in vivo diagnostic and regenerative medicine applications. Sean has developed novel multi-institutional courses in therapeutic cell manufacturing and leads CMaT REU, RET, and REM programs at UW-Madison.

In-hyun Park

(Yale University)

Dr. Park is an Associate Professor of Genetics, Yale Stem Cell Center, and Child Study Center. Dr. Park received his B.S and M.S. from Seoul National University at Korea, and Ph.D from University of Illinois at Urbana-Champaign in the field of Cell and Structural Biology. During his Ph.D training with Dr. Jie Chen, he studied mTOR pathways regulating cell growth, and myogenic differentiation. In 2005, he continued his research as a Post-doc fellow in Dr. George Daley’s lab in Children’s Hospital Boston, where he isolated one of the first human induced pluripotent stem cells (iPSCs). He started his own lab at Yale University from 2009. As an independent investigator, he pioneered in the generation of region-specific brain organoids, and study the human neurodevelopment and related disorders.

Francesco Pasqualini

(University of Pavia)

Francesco Pasqualini trained in biomedical engineering in Italy before moving to Boston (2010) and Zurich (2016) to study cardiovascular tissue engineering in the laboratories of Prof. Kit Parker (Harvard Wyss Institute) and Prof. Simon Hoerstrup (Wyss Translational Center). Francesco's research focuses on the development of in-silico and in-vitro quality assurance platforms for the characterization of stem cells in drug discovery, disease modeling, and regenerative medicine applications. In 2019, his Synthetic Matrix Biology project was awarded an ERC Stg grant to bring concepts from synthetic biology into the practice of tissue engineering. Since June 2020, Francesco is an associate professor in industrial bioengineering at the University of Pavia in Italy.

Jai Prakash

(University of Twente)

Jai Prakash is a Professor and Chair of Engineered Therapeutics at the Department of Advanced Organ bioengineering and Therapeutics at the University of Twente in The Netherlands. He is a pharmaceutical and entrepreneurial scientist with a background in developing novel targeted (nano)therapeutics against fibrosis and the tumor microenvironment. His research is highly multi-/inter-disciplinary integrating peptide technology, nanomedicine, as well as bioengineering fields for targeting cancer and fibrosis. His key research focuses on designing therapeutic technologies to disrupt the crosstalk between tumor cells and stromal cells and in developing advanced 3D in vitro models to mimic the tumor microenvironment.
He obtained PhD (cum laude) in 2006 from University of Groningen, The Netherlands in the field of drug targeting to treat renal fibrosis. Thereafter, he worked as a Vice President – Preclinical Research at BiOrion Technologies with a joint position at the University of Groningen. During this period, he developed several products, which are being translated by BiOrion. In 2011, he joined Karolinska Institutet in Stockholm (Sweden) as Assistant Professor in the Department of Oncology-Pathology. Later, he was invited to join as a Tenure-Track Professor at University of Twente to set up his new research line on novel therapeutics against fibrosis and cancer. In 2019, he joined the School of Engineering and Applied Sciences at Harvard University as a visiting professor for his sabbaticals. He has published >100 peer-reviewed publications in high impact journals including Advanced Materials, Science Advances, Trends in Cancer, Advanced Drug Delivery Reviews, etc. He is also a (co)-inventor on several international patent families. He has an editor and writer of a book on 'Tumor stoma: Biology and Therapeutics'. He is the founder and CSO of ScarTec Therapeutics, a spin-off company, focusing on the development of novel peptide therapeutics against fibrotic diseases and pancreatic cancer. He is serving as the President of Controlled Release Soceity (CRS) BeNeLux and France Local chapter. He is also the founder and chair of the International Conference on Nanomedicine meets the Tumor Microenvironment (NanoTME) which was organized for the first time in 2021 and a special issue is in progress in the journal Theranostics. He is the expert referee and/or panel member on more than 25 grant agencies including ERC, EPSRC, ANR, Inserm, NWO and FWO.

Gwendolen Reilly

(University of Sheffield)

Professor Gwendolen Reilly, DPhil, obtained her PhD in bone biomechanics from the University of York UK in 1998, supervised by one of our great, sadly departed, JMBBM colleagues - Professor John Currey. Since then, she conducted research in the fields of bone mechanobiology and then biomaterials and tissue engineering in institutions in Switzerland (ETH) and the US (Penn State, U. Penn and UIChicago). In 2004 she obtained her faculty position at the University of Sheffield UK where she is now a Professor in Musculoskeletal Bioengineering working at the Department of Materials Science and the INSIGNEO institute for in silico medicine. INSIGNEO works at the interface between medicine and computer modelling to better understand disease and treatment. Her research centres around two key themes; the effects of mechanical stimulation on differentiation and matrix formation by bone cells and the interactions between precursor bone cells and their biomaterial substrates. Recently her group has been focused on improving 3D tissue engineered models of bone to create humanised in vitro bone matrices that replicate important feature of bone matrix. We believe that accurate 3D models including bone's complex, multiscale, hierarchical structure are needed to facilitate more relevant bone disease research than allowed by 2D culture or animal models. Gwen has published 80+ papers and 6 book chapters in these areas. She is past president of the European Society for Biomechanics.
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Daniel Reumann

(Institute of Molecular Biotechnology Austria (IMBA))

Current workplace:
Institute of Molecular Biotechnology Austria (IMBA) of the Austrian Academy of Sciences (ÖAW)
Dr. Bohrgasse 3, 1030 Vienna, Austria

Professional experience and education:
PhD studies (06/2017- present): Modeling Human Brain Development in Cerebral Organoids
Knoblich Lab, IMBA, Vienna BioCenter PhD program, Austria
Thesis title: Connectomics in a Dish- Using cerebral organoids to model the developing dopaminergic reward circuit in vitro
Other projects:
2) development of a high throughput tissue clearing protocol for cerebral organoids
3) Development of a high throughput cryo-processing platform
MSc (09/2014-06/2017, passed with distinction): Molecular Biology (specialization Biochemistry)
University of Vienna, Vienna, Austria
Master thesis (Knoblich Lab, IMBA, Vienna, Austria): Disease Modeling in Cerebral Organoids- Development of an organoid fusion system for studying interneuron migration.
BSc (09/2011-07/2014, passed with distinction): Molecular Biotechnology
University of Applied Sciences (FH Campus Wien)
Bachelor thesis (Paulsen lab, University of Oslo, Oslo, Norway): Neurodevelopmental effects of phthalates and bisphenol A in chicken cerebellum and PC12 cells

Publications:
Broad applicability of a streamlined ethyl cinnamate-based clearing procedure. Masselink, W., Reumann, D., Murawala, P., Pasierbek, P., Taniguchi, Y., Bonnay, F., Meixner, K., Knoblich, J.A., and Tanaka, E.M. (2019). Development 146. DIO: 10.1242/dev.166884
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Detailed Cerebral Organoid Fusion Method. Bagley, J.A., Reumann, D., and Knoblich, J.A. (2017b). Protocol Exchange, DIO: 10.1038/protex.2017.064
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Elizabeth Rosado Balmayor

(RWTH Aachen University Hospital)

Elizabeth Rosado Balmayor is currently University Professor for Experimental Trauma Surgery and Mechanobiology at the RWTH Aachen University Hospital (Germany). She is the head of the newly created Teaching and Research Area “Experimental Orthopaedics and Trauma Surgery” at the Department of Orthopaedic, Trauma, and Reconstructive Surgery. She was trained as a Chemist and earned an M.Sc. in Materials Science and Technology at the University of Havana (Cuba). She received a Marie Curie scholarship in the area of Biomaterials and completed her Ph.D. in 2009 with Prof. Rui Reis at the 3B’s research group (Portugal). Elizabeth obtained her assistant professorship in Experimental Trauma Surgery from TUM (Germany) in 2017. Thereafter, she moved to the MERLN Institute for Technology-Inspired Regenerative Medicine at the Maastricht University (the Netherlands) where she was the Principal Investigator of the research group “Molecular Transfer and Therapy” until early 2022. She is an associate researcher with Prof. Chris Evans at the Mayo Clinic (USA) and holds visiting professorships at the Peruvian University Cayetano Heredia and the UNESCO Biomaterials chair of the University of Havana. Her achievements have been recognized with a number of awards and grants, including the Hans-Liniger-Award by the German Society for Trauma Surgery, and projects from the German Federal Ministry for Economic Affairs and Energy, the European H2020 funding scheme, and the U.S. National Institutes of Health - NIH. She has obtained over 3.5 million euros from competitive grants for her research.
A powerful breakthrough in Elizabeth’s research is the development of a chemically modified mRNA encoding BMP-2 as an alternative to traditional gene therapy for bone healing. She is a pioneer in the application of this novel technology to tissue regeneration and holds a patent on this discovery, which served as the basis for the creation of the start-up company Ethris GmbH of which she is one of the founders. Recently, the journal Science Advances published a compendium of Elizabeth’s results and the first demonstration of a critical-sized bone defect fully healed with chemically modified mRNA in a rodent model.
Elizabeth has published 63 publications in peer-reviewed scientific journals and authored 7 book chapters in books with international circulation. She is the author of 2 patents. In addition, she is associate editor of the European Journal of Medical Research and Frontiers in Bioengineering and Biotechnology sections Tissue Engineering and Regenerative Medicine and Preclinical Cell and Gene Therapy. She has been invited editor of special issues for the journals Advanced Drug Delivery Reviews, Tissue Engineering Part A, Frontiers, and International Journal of Molecular Sciences. Elizabeth has been a board member and young scientists’ representative at the

International Graduate School of Science and Engineering at TUM (Germany). She is currently the chair of the equal representation committee and ex-officio board member of the European Orthopaedic Research Society (EORS), and part of the communication committee of the Orthopaedic Research Society (ORS). She is part of the executive council of the European chapter of the Tissue Engineering and Regenerative Medicine International Society (TERMIS EU).

Antonio Salgado

(ICVS, School of Medicine, University of Minho)

António Salgado is a biologist with a PhD in Materials Science and Engineering-Tissue Engineering and Hybrid Materials (2005), and a Habilitation (DSc) in Health Sciences, from the University of Minho. Currently he is a Coordinating Investigator at the Life and Health Sciences Research Institute (ICVS), School of Medicine - University of Minho. His research interests are focused on the development of innovative therapies for CNS repair, namely on Spinal Cord Injury and Parkinson's Disease. His main areas of research are: 1) Development of ECM like hydrogels for the transplantation of Mesenchymal Stem Cells into the injured CNS; 2) Role of the secretome of MSCs in neuroprotection and repair, particularly the establishment of novel therapies based on the sole use of MSCs secretome. He is currently an author of more than 130 papers (over 6000 citations; h-Index=39, Clarivate Analytics Web of Science), and has delivery over 70 invited talks worldwide. He serves as the President of the Portuguese Society for Stem Cells and Cell Therapies, and as an Associate Editor for Biomed Research International, Biochimie, BMC Neuroscience and Stem Cells International. He has received several distinctions for his work including the Gulbenkian Award on Cutting Edge Research in Life Sciences, and the Prize Melo e Castro for Spinal Cord Injury Research, awarded by Santa Casa de Misericordia de Lisboa (2013, 2017 and 2021).

Arnaud Scherberich

(Department of Biomedicine, University of Basel)

Arnaud Scherberich has completed his PhD in December 1999 at the University of Strasbourg (France). Since 2007, he is leading a biomedical engineering research team at the University of Basel, Switzerland. His team investigates the biology and therapeutic potential of mesenchymal stromal cells for regenerative surgery applications. It involves the development of perfusion bioreactor-based models and the generation of pre-vascularized tissue grafts, primarily in the context of bone regeneration. In 2019, he became Adjunct Professor in Experimental Medicine. He has published more than 80 peer-reviewed articles and has an h-index of 30. Arnaud is an elected member of the Executive Committee of the Swiss Society for Biomaterials and Regenerative Medicine (SSB+RM) and he is member of the Scientific Board of 2 companies: Defymed (Strasbourg, France) and Cellec Biotek AG (Basel).

Romana Schirhagl

(Groningen University)

Romana Schirhagl graduated 2009 with a PhD in chemistry from Vienna University where she also studied biology as an additional subject. After that she did her postdoctoral work in physics at Stanford University (under Prof. Zare) and at ETH Zurich (under Prof. Degen). In 2014 she started her own group as Assistant Professor at Groningen University. In 2018 she was promoted to Associate Professor and in 2021 to Full Professor. Her research interests are in applying quantum sensing to biomedical applications. Among her biggest achievements is the first application of this method to measure the metabolic activity in living cells. During the course of her career she has been awarded several prestigious grants and awards including an INITs award, a Theodor Koerner prize and Ewald Wicke award and ERC starting grant or the Dutch VIDI grant. Further she has written over 100 scientific articles.

Günther Schlunck

(Eye Center - Freiburg University Medical Center)

Prof. Günther Schlunck is an ophthalmologist with a strong interest in cellular signal integration and mechanotransduction. Following his medical training and residency at the University of Freiburg, Germany, he gained postdoctoral research experience in the laboratory of Martin A. Schwartz at The Scripps Research Institute, La Jolla, U.S.A. As research group leader at Würzburg University Eye Hospital, he studied biomechanical aspects in ocular fibrosis and glaucoma. Since 2011, he is heading the Division of Experimental Ophthalmology at Freiburg University Medical Center in Freiburg, Germany.

Daniel Seitz

(BioMed Center Innovation gGmbH)

Daniel Seitz has studied biology in Frankfurt/M and Bayreuth, concluding his diploma thesis on the influence of Diplopods on nutrient fluxes in agroforestry systems in the amazon in Manaus, Brazil in 2003. He than changed into regenerative medicine, working in an engeering science-centered group at the Friedrich-Baur-Institute for Biomaterials close to Uni Bayreuth, with a focus on cartilage and bone tissue engineering. He has invented several bioreactors for in vitro culture and is part of a patent on a biosensory device. After several years in the stearing comittee, he took over the lead of the group in 2011, founding the non-profit organization BioMed Center Innovation gGmbH in 2012 on the base of former knowledge and equipment. He has been trained in regulatory and co-published on a suggestion to improve individual medical product registration. In the EU-project INKplant, he is responsible for realizing AM for clinical application and part of a team working on regulatory and standardization aspects.

Ólafur E. Sigurjónsson

(Reykjavik University)

Professor Ólafur E. Sigurjónsson holds a PhD in stem cell biology and immunology from the University of Oslo. He is a Professor at the school of engineering, Reykjavik University, program director in Biomedical engineering at same University and a clinical Professor at the department of medicine, school of health sciences, University of Iceland. Professor Sigurjonsson is the director of research and development at the Blood bank, Landspitali University Hospital, Iceland and the laboratory director for the clinical hematopoietic stem cell program at the same institute. He is the founder and CSO of Platome biotechnologies and is the former president of the Scandinavian Society for Biomaterials. Professor Sigurjonsson research group focuses research in tissue engineering of bone and adipose tissue, GMP culture of mesenchymal stem cells, platelet lysate development, systems biology and storage of blood components.

Cyrill Slezak

(Utah Valley University)

Cyrill Slezak is a computational physicist who received his doctoral degree in theoretical/computational condensed matter. Since then, his interests have evolved, and he now specializes in medical physics with an emphasis in regenerative physical therapies. Coming from a background in multiscale modeling he is applying computational tools in conjunction with in-vivo measurements to provide a new perspective on established therapies. He currently is the shockwave research group leader at the Ludwig Boltzmann Institute for Traumatology and a member of the physics department at Utah Valley University where he also serves as the director of the Institutional Review Board.

Martin Stoddart

(AO Research Institute Davos)

Prof. Martin Stoddart, FRSB is department head, responsible for the Regenerative Orthopaedics Program at the AO Research Institute Davos (ARI), Switzerland. His interests include the use of patient derived mesenchymal stem cells (MSCs) for bone and cartilage repair, in particular the use of bioreactors and the role of mechanoregulation during the initiation of MSC fate decisions. This has led to an increased understanding of chondrogenesis under complex physiological loads in the absence of exogenous growth factors. More recently he has been investigating markers of human MSC potency to identify sources of donor variation and investigating osteogenic differentiation to identify off target effects.
He completed his bachelors in Biology in 1995 and M.Phil in 1996 at the University of Aberystwyth, Wales, UK. He completed his doctoral thesis in Oncology (University of Nottingham, UK) in 1999. In 2000 he moved to the Laboratory for experimental cartilage research, Zürich, Switzerland, initially as Post-Doc and from 2003 as Group Head, moving to ARI in 2005. In 2002 he took a sabbatical at the Centre for Molecular Orthopeadics, Harvard Medical School, Brigham and Womens Hospital, Boston, to learn viral gene transfer techniques. He was awarded a Professorship from at the Medical Faculty of the Albert-Ludwigs University, Freiburg, Germany in 2015 and an Honorary Professorship from Keele University, UK in 2016. In 2016 he

was elected Fellow of the Royal Society of Biology and Fellow Member, International Cartilage Research Society (ICRS). In 2020 he was recipient of the TERMIS-EU mid-term Career Award. He is TERMIS EU Member at Large, Chair of LearnORS, editor on multiple journals, including eCM Journal, and is the conference chair of the yearly eCM Conference. He is a member of the ICORS Steering Committee and the International Consortium for Regenerative Rehabilitation Leadership Council. He is the author of over 140 scientific papers and book chapters with a H index of 39 and is the editor of 2 books.

Yasuhiko Tabata

(Institute for Frontier LIfe and Medical Sciences, Kyoto University)

Dr. Yasuhiko Tabata is the Professor and Chairman of the Laboratory of Biomaterials at the Institute for Frontier Life and Medical Sciences, Kyoto University and a Professor of the Graduate School of Medicine, Osaka University, and guest professors at the Graduate School of Medicine, Dentistry, Pharmaceutical Sciences, and Engineering of 17 different universities. He received his BD in Polymer Chemistry (1981), Ph.D. (1988) in Technology, D.Med.Sc. (2002), and D.Pharm. (2003) all at Kyoto University. He was a Visiting Scientist at the MIT (Professor Robert Langer) (1991-92). He has published 1,710 scientific papers including 146 book chapters and review articles, and has 130 patents. He received the Young Investigator Award (1990), the Scientific Award from the Japanese Society for Biomaterials (2002), the Scientific Award from the Japan Society of Drug Delivery System (2011), Chandra P. Sharma Award of the International Society of Biomaterials & Artificial Organs (2011), the Scientific Award from the Japanese Society for Regenerative Medicine (2014), Merit Award Winners for Industry-Academia-Government Collaboration, President of Science Council of Japan Award (2016), Outstanding Scientist Award of the Tissue Engineering and Regenerative Medicine International Society - Asian-Pacific Chapter (TERMIS-AP) (2018), and several awards.
Dr. Tabata is the board member of the Japanese Society of Regenerative Medicine (JSRM), the Japanese Society for Biomaterials (JSB), the Japan Society of Drug Delivery System (JSDDS), and the Japanese Society of Inflammation and Regeneration (JSIR) or the councilor of the Japanese Society of Wound Healing, the Japanese Artificial Organ Society, He is an associate member of the Science Council of Japan, Cabinet Office, a fellow of the World Biomaterials Society (WBS), the TERMIS, the Controlled Release Society (CRS) or the New York Academy of Science and American Institute for Medical and Biological Engineering (AIMBE), and the Founding Fellow for Tissue Engineering and Regenerative Medicine, (FTERM).
Dr Tabata is the one of founder members of Asian Biomaterial Federation (ABF). He is a board member of Tissue Engineering Society International for 2001-2003 and 2012-present. He organized as the chairman the 13th Annual Congress of JSRM (2014) and the 37th Annual Congress of JSB (2015), the 37th Annual Congress of JSIR (2016), and the 33th Annual Congress of JSDD (2017). He is a board member of TERMIS-AP for 2013-present and the president of TERMIS-AP for 2016-2020, and chairs the 5th TERMIS World Congress 2018, Kyoto, Japan.
His research is very interdisciplinary in nature and brings together the fields of polymer chemistry, pharmaceutical science, biology, and basic and clinical medicines. He actively proceeds translational researches by linking bioengineering scientists, clinicians, and industry to achieve clinical therapies of regenerative medicine as well as produce some commercialization products for basic researches and cosmetics. His research focuses on the design and preparation of biodegradable or non-biodegradable biomaterials for their biological, medical, and pharmaceutical applications, while the keywords are biomaterials, drug delivery system (DDS), tissue engineering, regenerative medicine, stem cell technology, and medical diagnostics.

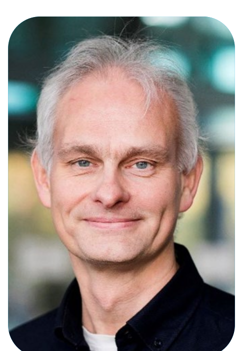
Andreas Traweger

(Paracelsus Medical University)

Andreas Traweger currently holds a Research Professorship for Regenerative Biology at the Paracelsus Medical University in Salzburg, Austria. He received his PhD in Genetics from the University of Salzburg and completed his post-doctoral training at the Samuel Lunenfeld Research Institute in Toronto, Canada. He was then R&D Manager at Baxter (Vienna, Austria) for 4 years before joining Paracelsus Medical University in 2012.
His research is interdisciplinary in nature, focusing on both fundamental science and translation for human health. He published numerous peer-reviewed manuscripts, including articles in Cell, PNAS, and Scientific Translational Medicine, holds a patent application, and serves as ad hoc reviewer for a number of renowned journals and funding bodies. His interests lie in promoting the understanding of tendon and bone biology in general and to devise novel strategies to improve tendon and ligament healing. To achieve this goal, he works together with an interdisciplinary team of biologists, veterinarians, engineers, and trauma surgeons. He has a considerable track record in the research area of tendinopathy, focusing on the role of neoangiogenesis, tissue and cell senescence, and inflammatory processes in tendon health and disease.

Jan Van Hest

(Eindhoven University of Technology)

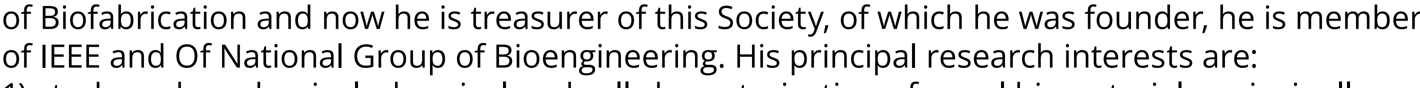


Biotext:
Jan van Hest obtained his PhD from Eindhoven University of Technology in 1996 in macro-organic chemistry with prof E.W. Meijer. He worked as a postdoc with prof D.A. Tirrell on protein engineering. In 1997 he joined the chemical company DSM in the Netherlands. In 2000 he was appointed full professor in Bio-organic chemistry at Radboud University Nijmegen. As of September 2016 he holds the chair of Bio-organic Chemistry at Eindhoven University of Technology. Since May 2017 he is the scientific director of the Institute for Complex Molecular Systems (ICMS). The group's focus is to develop well-defined compartments for nanomedicine and artificial cell research. Using a combination of techniques from polymer science to protein engineering, well-defined carriers and scaffolds are developed for application in e.g. cancer treatment, immunology and ophthalmology.
Van Hest was elected member of the "Jonge Akademie", (The Young Academy of the Royal Netherlands Academy of Arts and Sciences) in 2005. He has won a TOP grant (2007) and a VICI grant (2010), the highest personal career grant obtainable through open competition in the Netherlands. In 2012 he was one of the main applicants of the 10-year gravitation program on functional molecular systems (26.9 M€, with the universities of Eindhoven, Groningen and Nijmegen). In 2016 he was awarded an ERC Advanced grant. Van Hest is associate editor of Bioconjugate Chemistry. He is furthermore an advisory board member of Macromolecular Bioscience, Biomacromolecules, Journal of Materials Chemistry, Chemical Science and ACS Central Science. He has been elected member of the Academy of the Royal Netherlands Academy of Arts and Sciences in 2019, and was awarded the Spinoza premium in 2020, the highest scientific distinction in the Netherlands He has published around 400 papers. He is also cofounder of four start-up companies (Encapson, FutureChemistry, Noviosense and Noviotech).

Shyni Varghese

(Duke University)

Shyni Varghese, Ph.D., is a Professor of Biomedical Engineering, Mechanical Engineering & Materials Science, and Orthopaedics Surgery at Duke University. She is the inaugural MEDx Investigator at Duke University. Prior to moving to Duke, she was a Professor of Bioengineering at University of California, San Diego. Dr. Varghese's research covers a broad range of topics including stem cells, biomaterials, biologically inspired systems, tissue chips, and regenerative medicine. Her research activities have resulted in over 100 publications and over a dozen patent disclosures. Examples of ongoing research activities in her laboratory involve developing functional biomaterials such as self-healing hydrogels and biomineralized matrices; technologies to improve cell-based therapies including stem-cell differentiation, cell transplantation, activating endogenous stem cells, and engineered functional tissue grafts; and organ-on-a-chip technologies. She is a fellow of AIMBE and Royal Society of Chemistry. She is currently serving as an Associate Editor of Biomaterials Science (an RSC journal).



Giovanni Vozzi

(Research Center "E. Piaggio" & Department of Ingegneria dell'Informazione, University of Pisa,)

Giovanni Vozzi was graduated in Electronic Engineering in 1998 at the University of Pisa. In 2002 he got the PhD in Bioengineering at Polytechnical of Milan. At present he is a full professor in Bioengineering. He acts as supervisors of PhD students in Materials Engineering and Biomedical Engineering and coordinates the work of several undergraduate students and graduate fellows. He was member of Board of Directors of International Society of Biofabrication and now he is treasurer of this Society, of which he was founder, he is member of IEEE and Of National Group of Bioengineering. His principal research interests are:
1) study and mechanical, chemical and cell characterization of novel biomaterials, principally obtained by waste materials and design of new methodologies of their functionalisation for application to Biomedical Engineering and to several industrial area;
2) development of new micro and nanofabrication techniques for application to Biomedical Engineering and to several industrial area;
3) design and development of novel biofabrication approaches for in vitro tissue model development;
3) development of in vitro tissue model and of innovative bioreactors
4) in silico cell modelling.
These scientific activities are demonstrated by more than 150 papers on International journal with high IF (h-index=34) 12 chapters on book and 24 patents.
He is Editorial Board member of several journal and acts as reviewers for the most important journal in his research field. He was involved and coordinator in several national and european research projects.

Wenxin Wang

(University College Dublin)

Wenxin Wang is Full Professor in Skin Research and Wound Healing, a Science Foundation Ireland (SFI) Principal Investigator at the Charles Institute of Dermatology, School of Medicine, University College Dublin (UCD), and a member of UCD Academic Council. Prof Wang is Senior Conway Fellow at UCD Conway Institute of Biomolecular and Biomedical Research in UCD; Adjunct Professor at School of Mechanical and Materials Engineering in UCD; and Guest Professor at Department of Polymer Science and Engineering in Zhejiang University (Hangzhou, China). He won the highly prestigious "Young Scientist Prize in Regenerative Medicine" in 2010 at TERMIS-EU conference, the "SFI Principal Investigator award" in 2011 and the DEBRA Award for Excellent EB Patient Service in 2014, which highlight his work ethic and achievements. Prof. Wang's scientific interests are in the areas of biomaterials, stem cell and gene therapy for the treatments of skin wounds, cartilage/bone regeneration, dental tissue regeneration, tissue sealant/adhesive. His scientific contribution and achievements have been recognized both nationally and internationally including over 210 peer-reviewed scientific journal papers (Nat. Commun., Nat. Rev. Chem., Sci. Adv., Angew. Chem., JACS, Chem. Sci. and Nano Letters etc.), 5 book chapters, 34 patents, 140 conference abstracts and presentations, and 113 invited lectures and keynote presentations. Since 2009 he has graduated 19 PhD students and mentored over 25 postdoctoral researchers. His achievements have gained the increased interest in the wider public community with publicity media activities (56 times in TV Documentary, Videos and Newspapers), for example in RTE-TV, 'The Sunday Times', 'The Irish Times', 'Science Daily' and 'Chemistry World'. He has been awarded significant funding (ca. 11.4 million Euros) from different sources, e.g., SFI, Health Research Board (HRB), Irish Research Council (IRC), Enterprise Ireland (EI) and European Union (EU-FP7 & EU Horizon 2020) to support his research activities. Prof. Wang has acted as the symposium convener and chair, the member of organizing committees and the member of the conference advisory board for 31 international conferences and has been selected as an expert reviewer and panel member by 26 international research councils and funding bodies. As the founder, Prof Wang has launched 3 companies - Vornia Ltd (acquired by Ashland - a Fortune500 US company in Jan. 2018, renamed as Ashland Specialties Ireland), Blafar Ltd., Branca Bunús Ltd. Furthermore, he has licensed 17 new technologies to 5 companies: Ashland, Amryt Biopharm, Blafar, Ruixi, and Branca Bunús, and successfully launched and commercialized newly developed technologies onto the market.

Thomas Webster

(Hebei University of Technology)

Thomas J. Webster's (H index: 109; Google Scholar) degrees are in chemical engineering from the University of Pittsburgh (B.S., 1995) and in biomedical engineering from RPI (Ph.D., 2000). He served as a professor at Purdue (2000-2005), Brown (2005-2012), and Northeastern (2012-2021) Universities and has formed over a dozen companies who collectively have over 23 FDA approved medical products currently in thousands of patients. He currently a Professor at Hebei University of Technology, Vellore Institute of Technology, and Universidade Federal do Piauí (UFPI). He is also currenting directly numerous spin-out companies. He has directed numerous international centers in biomaterials and has graduated over 200 students with over 750 peer-reviewed publications. Prof. Webster is a fellow of over 8 academic societies. He is a Clarivate 0.1% Most Distinguished Researcher in Citations (Pharmacology and Toxicology), a SCOPUS highly cited researcher (top 1% citations for materials

science and mixed fields), and a Public Library of Science (PLoS) World Top 2% Scientist by Citations in all fields.

Britt Wildemann

(Experimental Trauma Surgery; Department of Trauma, Hand and Reconstructive Surgery; Jena University Hospital)

Prof. Dr. Britt Wildemann is a biologist and obtained her PhD in neurobiology from the Freie Universität Berlin, Germany in 1998. In 1999 she started her research on musculoskeletal regeneration at the Charité-Universitätsmedizin Berlin and has been a professor in the Berlin-Brandenburg Center for Regenerative Therapies (BCRT) since 2009. In June 2018 she became head of the "Experimental Trauma Surgery" at the Jena University Hospital, Germany. Her research investigates the regeneration of the musculoskeletal system with the two main interests: 1. Osseous regeneration and infection prophylaxis and treatment and 2. Tendinopathy and tendon regeneration. The projects are funded by various national and international grants and she was awarded with several prizes including COPP-Award 2004 of the German Society of Osteology, APOA-Pfizer Best Scientific Paper Award 2011 for Orthopaedic Infection, and Oskar-Helene-medical award 2011 of the Oskar-Helene-Heim Foundation. She is member of the editorial review board for several journals: e.g. Bone and Joint Research, eCM, International Journal Molecular medicine, Journal of Orthopaedic Research and Scientific Reports and member of the relevant scientific organizations. Britt is the German ambassador for the eORS.

Michał Wszoła

(Polbionica)

- Chairman of the Scientific Council of the Foundation of Research and Science Development
- CEO at Polbionica sp. z o.o.
- Medical director of CM Medispace

A transplant surgeon, dedicated physician and visionary scientist, originator of the bionic pancreas – an organ that will restore the body's ability to regulate blood sugar and revolutionize the treatment of diabetes. He has been running a medical practice for years, leading a team that conducts innovative research - on a global scale – and searches for innovative solutions in medicine. He participated in the first pancreatic islet transplant in Poland (2008), the first pancreas-only transplant (2010) and the first exchange of kidney pairs between family donors (2015). The combination of his passions, transplantology and endoscopy, led to the creation of a new method of treating diabetes – endoscopic transplantation of pancreatic islets under the gastric mucosa. In 2013, he performed first such procedure in the world. He is currently continuing to work on a 3D printing project of a bionic pancreas with a research team at the Foundation of Research and Science Development. Since 2017, the Foundation, as the leader of the Bionic Consortium, has been working on the implementation of the bionic pancreas project. After only 2 years of preparation and testing, the world's first vascularized bionic pancreas prototype measuring 3x3x5cm was printed. Prof. Wszoła is also a co-founder of the medical knowledge sharing portal medtube.net and the medical app medizzy.com. Medizzy brings together over 400,000 doctors and medical students from around the world. The community of portal medtube.net in 2021 exceeded 250,000+ professional subscribers and the library reached 25,000+ medical materials.

Ozlem Yesil-Celiktas

Ozlem Yesil-Celiktas is a Chemical Engineer from Ankara University, with an M.Sc from Chalmers University of Technology in Sweden and a PhD in Biotechnology from Ege University, Izmir. She has six years of industrial experience with international secondments in Munich, Stockholm and Wageningen. Prof. Dr. Yesil-Celiktas is working as a faculty member at the Bioengineering Department, Ege University since October, 2007. She had been a visiting Assoc. Prof. Dr. at Brigham and Women's Hospital, Harvard Medical School in 2017. Currently, she leads Biomimetic Microsystems research group, which applies a multidisciplinary approach to design and develop organ-on-chip platforms that enable to elucidate cellular and molecular mechanisms governing tissue pathology or offering protection during injury. Her research lies at the intersection of neuronal and pulmonary diseases, immuno-microbiology, tissue engineering, and biomaterials. Ultimately, her goal is to discover novel drug targets and personalized diagnostics using microengineered systems that recapitulate complex human organ pathophysiology in vitro. She thrives to positively impact human health and our society through discoveries.

David Young

(Newcastle University)

CURRICULUM VITAE – David YOUNG
Nationality: British
Contact: d.a.young@ncl.ac.uk, Skeletal Research Group, Biosciences Institute, Centre for Life, Central Parkway, Newcastle University, Newcastle upon Tyne, NE1 3BZ
Research Interests
My group are based in the Biosciences Institute, Newcastle University. Our current research is focussed on understanding and modulating gene regulation of chondrocytes and molecular programmes that control their fate including their dysregulation in disease in vitro and in vivo; continuing a long-standing research interest centred on gene regulation. A recent contribution of the group has been in the identification and understanding of the 'epigenetic' mechanisms by which chondrocytes and cartilage are regulated, which includes microRNAs, short non-coding RNAs that post-transcriptionally regulate gene expression.
Current position
Non-clinical Professor of Musculoskeletal Biology, Biosciences Institute. HEFCE funded
Recent relevant publications.

1. Young DA, Barter MJ, Soul J. Osteoarthritis year in review: genetics, genomics, epigenetics. Osteoarthritis Cartilage. 2022;30(2):216-25.
2. Wilkinson DJ, Falconer AMD, Wright HL, Lin H, Yamamoto K, Cheung K, et al. Matrix metalloproteinase-13 is fully activated by neutrophil elastase and inactivates its serpin inhibitor, alpha-1 antitrypsin: Implications for osteoarthritis. FEBS J. 2022;289(1):121-39.
3. Chaudhry N, Muhammad H, Seidl C, Downes D, Young DA, Hao Y, et al. Highly efficient CRISPR-Cas9-mediated editing identifies novel mechanosensitive microRNA-140 targets in primary human articular chondrocytes. Osteoarthritis Cartilage. 2022.
4. Stadnik PS, Gilbert SJ, Tarn J, Charlton S, Skelton AJ, Barter MJ, et al. Regulation of microRNA-221, -222, -21 and -27 in articular cartilage subjected to abnormal compressive forces. J Physiol. 2021;599(1):143-55.
5. Patel K, Chandrasegaran S, Clark IM, Proctor CJ, Young DA, Shanley DP. TimiRGeN: R/

Bioconductor package for time series microRNA-mRNA integration and analysis. Bioinformatics. 2021.
6. Jones TL, Esa MS, Li KHC, Krishnan SRG, Elgallab GM, Pearce MS, et al. Osteoporosis, fracture, osteoarthritis & sarcopenia: A systematic review of circulating microRNA association. Bone. 2021;152:116068.
7. Hayman DJ, Modebadze T, Charlton S, Cheung K, Soul J, Lin H, et al. Increased hippocampal excitability in miR-324-null mice. Sci Rep. 2021;11(1):10452.
8. Barter MJ, Cheung K, Falk J, Panagiotopoulos AC, Cosimini C, O'Brien S, et al. Dynamic chromatin accessibility landscape changes following interleukin-1 stimulation. Epigenetics. 2021;16(1):106-19.
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Maximina Yun

(CRTD-Center for Regenerative Therapies Dresden & Max Planck Institute for Molecular Cellular Biology & Genetics)

Education:

2009	PhD in Biological Sciences, University of Cambridge and MRC - Laboratory of Molecular Biology (LMB), UK
2004	BSc Biological Sciences, University of Buenos Aires, Argentina
	Positions held
since 2017	Group Leader, CRTD and MPI-CBG
2016-2017	Independent Senior Research Associate, University College London, UK
2014-2016	Senior Research Associate, Institute of Structural and Molecular Biology, University College London, UK
2009-2014	Postdoctoral Research Associate, Institute of Structural and Molecular Biology, University College London, UK
2009	Investigator Scientist, MRC-Laboratory of Molecular Biology of Cambridge and Cambridge University, UK
2005-2009	PhD research, MRC-Laboratory of Molecular Biology of Cambridge and Cambridge University, UK
2002-2005	Undergraduate research, Institute of Biochemical Research of Buenos Aires - Foundation Institute Leloir, Argentina

Honors:

2021	TUD Young Investigator
2017	MPI-CBG Fellow
2016-2018	UCL-IHA Associate Fellow
2009-2011	UCL-Wellcome Trust VIP Fellowship
2008	Max Perutz Prize for outstanding PhD studies, MRC-LMB Cambridge
2005-2008	Cesar Milstein Scholarship, MRC-LMB Cambridge
2004-2005	Start to research Undergraduate Scholarship, University of Buenos Aires
2002-2003	Paulo Barroso Mastronardi Honor Scholarship in Biological Sciences. National prize for highest academic achievement. National Academy of Exact, Physical and Natural Sciences, Argentina.

Yu Shrike Zhang

(Harvard Medical School)

Dr. Zhang is an Assistant Professor in the Department of Medicine at Harvard Medical School and Associate Bioengineer in the Division of Engineering in Medicine at the Brigham and Women's Hospital. He is directing the Laboratory of Engineered Living Systems, where the research is focused on innovating medical engineering technologies, including 3D bioprinting, organs-on-chips, microfluidics, and bioanalysis, to recreate functional tissues and their biomimetic models, for applications in regenerative medicine and personalized medicine. Dr. Zhang is an author of ~280 peer-reviewed publications (>45 covers; citations >22,000, h-index = 74). His scientific contributions have been recognized by >45 international, national, and regional awards, notable ones including ACS Kavli Emerging Leader in Chemistry Lecture (2022), Materials Today Rising Star Award (2020), C&EN Talented 12 (2018), Society for Mechanical Engineers Marcus Crotts Outstanding Young Manufacturing Engineer Award (2018), IEEE Sensors Council Technical Achievement Award (Early Career) (2018), TERMIS-AM Young Investigator Award (2018), and the Lush Prize—Young Researcher (Americas) (2016).

Plenary Sessions

CARTILAGE REGENERATION: THE CHALLENGES OF REGENERATING A “SIMPLE” NON-VASCULARISED TISSUE

Room: S1 (29 Jun 2022, 09:00 - 10:00)
Conveners: Geoff Richard
Presenter: VAN OSCH, Gerjo

ENGINEERING IN PRECISION MEDICINE

Room: S1 (29 Jun 2022, 17:30 - 18:30)
Conveners: Jos Malda
Presenter: KHADEMHOSSEINI, Ali

Engineering in Precision Medicine

Abstract: Engineered materials that integrate advances in polymer chemistry, nanotechnology, and biological sciences have the potential to create powerful medical therapies. Dr. Khademhosseini is interested in developing 'personalized' solutions that utilize micro- and nanoscale technolgoies to enable a range of therapies for organ failure, cardiovascular disease and cancer.In enabling this vision he works closely with clinicians (including interventional radiologists, cardiologists and surgeons). For example, he has developed numerous techniques in controlling the behavior of patient-derived cells to engineer artificial tissues and cell-based therapies. His group also aims to engineer tissue regenerative therapeutics using water-containing polymer networks called hydrogels that can regulate cell behavior. Specifically, he has developed photo-crosslinkable hybrid hydrogels that combine natural biomolecules with nanoparticles to regulate the chemical, biological, mechanical and electrical properties of gels. These functional scaffolds induce the differentiation of stem cells to desired cell types and direct the formation of vascularized heart or bone tissues. Since tissue function is highly dependent on architecture, he has also used microfabrication methods, such as microfluidics, photolithography, bioprinting, and molding, to regulate the architecture of these materials. He has employed these strategies to generate miniaturized tissues. To create tissue complexity, he has also developed directed assembly techniques to compile small tissue modules into larger constructs. It is anticipated that such approaches will lead to the development of next-generation regenerative therapeutics and biomedical devices.

BIOPRINTING 3D VASCULARIZED TISSUE FLAPS

Room: S1 (30 Jun 2022, 09:00 - 10:00)
Conveners: Lorenzo Moroni
Presenter: LEVENBERG, Shulamit

Bioprinting 3D vascularized tissue flaps
Shulamit Levenberg
Faculty of Biomedical Engineering, Technion, Israel

Engineering vascularized constructs represents a key challenge in tissue engineering. Sufficient vascularization in engineered tissues can be achieved through coordinated application of improved biomaterial systems with proper cell types. We have shown that vessel network maturity levels and morphology are highly regulated by matrix composition and analyzed the vasculogenic dynamics within the constructs. We also explored the effect of mechanical forces on vessels organization and demonstrated that morphogenesis of 3D vascular networks is regulated by tensile forces. Revealing the cues controlling vascular network properties and morphology can enhance tissue vascularization and improve graft integration prospects. Creating complex vascular networks with varying vessel sizes is the next challenge in engineering vascularized tissue flaps. 3D bioprinting, the controlled and automatized deposition of biomaterials and cells, represents a very attractive approach to solve this issue. This technique allows for combining different bioinks in an organized fashion to attain native-tissue mimicking structures. We showed that bioprinting macro-vessels with self-assembled micro-vessels allows fabrication of multi-scale vascular networks.

COMMENTATIO HISTORICA ET PHILOLOGICA - PERSPECTIVES AND CHALLENGES IN REGENERATIVE MEDICINE

Room: S1 (1 Jul 2022, 09:00 - 10:00)
Conveners: Manuela E. Gomes
Presenter: HUTMACHER, Dietmar W.

Despite the success of a handful of companies and the overabundance of research the tissue engineering & regenerative medicine (TE&RM) community has still not delivered the more then 3 decades ago promised health care and commercial break through's. Yet, rendition of research outcomes into clinical application is still the rallying cry of the modern TE&RM establishment. Deciphering observations from basic investigation protocols (e.g., in silico, in vitro, ex vivo, or in vivo, ,etc.) to address into not even clinical routines but first in human studies has diverse challenges. Exploitation of the vast literature uncritically to defend further research, regardless of their authenticity, clinical significance, vigour or quality, is often the most hurried path to write and fund grant proposals and to obtaining publishable data, even though these data inherit no translational and often little scientific relevance. The research data manufacturing machinery is authorised but real world applicability and impact are often compromised. Muted translational achievements continue to plague most aspects of TE&RM research as the development of bedside to bench and back again approach possess challenges that most academics but also TE&RM companies are not typically trained to overcome. Based on the above arguments this talk will critically review two key research areas in TE&RM, namely scaffold guided tissue engineering and bioprinting.

S01
3D in vitro tissue-engineered cancer/disease models
Session I - Room: S1
(28 Jun 2022, 11:00 - 12:30)

Conveners: Anna-Dimitra Kataki; Silvia Farè

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BIOENGINEERED PLATFORM TO STUDY IMMUNE-CANCER CELL INTERACTIONS EX VIVO

Shyni Varghese (Duke University, Durham, United States)

Cancer is a complex disease system in which the extracellular microenvironment provides robust physicochemical cues to promote disease progression and resistance to therapy. Immunotherapies have been considered as an efficient therapeutic strategy to treat cancer and are being studied for their potential to improve prognosis and long-term survival of patients. While early clinical data shows the potential of immune therapy in treating liquid cancers, emerging evidence highlight the influence of microenvironmental factors on determining the efficacy of such therapeutic strategies to treat solid tumors. A detailed understanding of the interdependency between the microenvironment and cancer/immune cell interactions is needed to enable the efficient use of immunotherapy to treat solid cancers. Because of limitations inherent to existing model systems, development of advanced in vitro platforms including tumor microenvironment with immune cells are needed. In this talk, I will describe our efforts to create ex vivo platforms such as tumor-on-chip to study cancer cell-immune cell interactions within a tumor specific microenvironment.

keywords: Cancer, organoid, microenvironment

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MODELLING BREAST-TO-BONE METASTATIC MECHANISMS VIA MICROFLUIDIC BIOFABRICATION

Gianluca Cidonio (Italian Institute of Technology, Rome, Italy), Virginia Brancato (Italian Institute of Technology, Milan, Italy), Chiara Scognamiglio (Italian Institute of Technology, Rome, Italy), Matteo Marzi (Italian Institute of Technology, Milan, Italy), Andrea Barbetta (University of Rome La Sapienza, Rome, Italy), Mara Riminucci (University of Rome La Sapienza, Rome, Italy), Francesco Nicassio (Italian Institute of Technology, Milan, Italy), Giancarlo Ruocco (Italian Institute of Technology, Rome, Italy)

Bone is a frequent homing site for breast cancer metastasis. Unfortunately, the majority of patients diagnosed with primary breast cancer is often affected by bone metastasis from where cancer can reach other vital organs such as the lungs. Thus, it is vital that the process of metastatic attraction and homing is modeled in vitro, harnessing tissue engineering technology, generating viable models that would be able to closely mimic the in vivo homing mechanism.
We engineered a three-dimensional breast-to-bone model using a novel microfluidic biofabrication approach by depositing human bone marrow stromal cells (hBMSCs) and a highly aggressive, invasive and poorly differentiated triple-negative breast cancer cell line (MDA-MB-231). Initially, the co-culture conditions have been optimised in order to preserve the viability of both cell types over 21-days of culture. Material inks were designed to grant the robust differentiation of HBMSCs and aid MDA-MB- 231 migration in 3D. A novel nanoclay-based material, comprising a variable ratio of alginate and gelatin, was used to encapsulate HBMSCs and investigate viability and functionality, while MDA-MB-231 were included in an RGD-based material to support cell migration and proliferation in 3D.
A metastasis-colonisation model was 3D printed and investigated for ultimate functionality. HBMSC- laden nanoclay-based ink was deposited in 3D following a lattice architecture with porosity augmented over a single planar direction, followed by the inclusion of MDA-MB-231-laden matrix on the most- top model surface, allowing the penetration and colonisation of the bone tissue.
In conclusion, we demonstrate the functionality of a 3D printed breast-to-bone model able to recapitulate the complex skeletal secondary site, illustrating the potential as a testing platform for novel therapeutic agents for metastatic process studies.

keywords: Metastasis, Biofabrication, Bone

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INDUCTION OF BRANCHING MORPHOGENESIS IN CHOLANGIOCARCINOMA ORGANOIDS IN VITRO IMPROVES SIMILARITY WITH THE ORIGINAL TUMOR FOR ENHANCED PERSONALIZED MEDICINE APPLICATIONS

Gilles Van Tienderen (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Kathryn Monfils (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Floris Roos (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Luc Van der Laan (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Monique Verstegen (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands)

Background and Aims: Cholangiocarcinoma (CCA) is an aggressive, heterogeneous cancer with low survival rates. Patient-derived cholangiocarcinoma organoids (CCAOs) hold potential for understanding disease progression and developing novel treatment options, based on their ability to mimic the original tumor. However, a hallmark of cancer is the disturbance of morphological cues resulting in typical dysplastic tumor architecture, an aspect not well recapitulated in CCAOs. Currently, CCAO expansion protocols focus on stimulation of the canonical WNT/β-catenin pathway, however there is growing evidence that non-canonical WNT pathways also play a crucial role in cancer progression. This project aims to establish a novel in vitro 3D model for CCA, better recapitulating the in vivo tumor, by stimulating both canonical and non-canonical WNT pathways.

Method: Branching cholangiocarcinoma organoids (BRCCAOs) ($n = 3$ patients) were established with a two-step protocol. First, CCAOs were initiated and cultured under standard conditions in canonical WNT stimulating expansion medium. Next, expansion medium was replaced by medium that stimulates canonical WNT-signaling (through R-spondin) and non-canonical WNT-signaling (with Dickkopf-related protein 1, DKK1) simultaneously, after which a branching-like morphology could be observed. Tumor cell behavior in BRCCAOs and CCAOs was assessed and compared through immunohistochemical stainings, bulk RNA-sequencing, and drug response studies.

Results: BRCCAOs presented a distinct branching morphology, with the formation of peripheral branches with a lumen, variable in diameter, surrounded by compact layers of cells. This morphology displayed an architecture similar to in vivo tumors, while maintaining tumorigenic potential and showing a lack of defined cellular polarity through staining of zonula occludens-1 (ZO-1). Bulk RNA-sequencing of BRCCAOs unveiled significant upregulation of cancer-associated molecular pathways, including hypoxia, compared to CCAOs and a close correlation (coefficient 0.80 ± 0.05) to the transcriptome of the original tumor tissue. BRCCAOs also exhibited patient-specific responses to a large panel of 166 anti-cancer drugs, including a universal strong resistant phenotype to multiple drugs that have previously failed in clinical trials for CCA patients (i.e. docetaxel, palbociclib, and irinotecan). Specifically, compared to CCAOs, BRCCAOs showed an approximately 10.000-fold ($p < 0.0001$) increase in chemo resistance against gemcitabine and cisplatin, first-line chemotherapy drugs for CCA, independent of patient variance.This resistant phenotype mimics patient response as clinically CCA patients experience only a modest increase in overall survival when receiving gemcitabine and cisplatin combinational therapy.

Conclusion: These results demonstrate that BRCCAOs better resemble in vivo CCA tumor tissue compared to conventional CCAOs, particularly with regards to morphology, transcriptome profile, and drug responses. Gemcitabine and cisplatin combinational therapy only provides CCA patients with a modest benefit in overall survival, and BRCCAOs appear to mimic this response more closely. This fosters new possibilities for personalized medicine applications.

keywords: Tumor organoids, morphogenesis, cholangiocarcinoma, personalized medicine

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A TUMOUR MICROENVIRONMENT MODEL FOR PANCREATIC CANCER

Verena Kast (Leibniz Institute of Polymer Research Dresden e.V., Max Bergmann Center of Biomaterials, Dresden, Germany), Anna Taubenberger (Center for Molecular and Cellular Bioengineering, BIOTEC, Technische Universität Dresden, Dresden, Germany), Ali Nadernezhad (Leibniz Institute of Polymer Research Dresden e.V., Max Bergmann Center of Biomaterials, Dresden, Germany), Hauser Sandra (Department of Radiopharmaceutical and Chemical Biology, Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany), Dagmar Pette (Leibniz Institute of Polymer Research Dresden e.V., Max Bergmann Center of Biomaterials, Dresden, Germany), Carsten Werner (Leibniz Institute of Polymer Research Dresden e.V., Max Bergmann Center of Biomaterials, Dresden, Germany), Daniela Loessner (Leibniz Institute of Polymer Research Dresden e.V., Max Bergmann Center of Biomaterials, Dresden, Germany)

Introduction: Pancreatic cancer is a devastating malignancy, and treatment options are very limited [1]. Progression of the disease and resistance to therapy are mediated by the tumour microenvironment (TME), which is composed of excessive amounts of extracellular matrix proteins, as well as stromal and immune cells, acting as a physical barrier for drug delivery [1-3]. Cell-secreted factors, such as kallikrein-related peptidases (KLKs), are important players in the TME [3]. Elevated expression of KLK6 is associated with poor survival rates in pancreatic cancer, making it an attractive target for alternative treatment strategies [3]. There is a lack of pre-clinical models of pancreatic cancer that reconstruct different elements of the TME to study disease progression and response to treatment. To address this limitation, and to explore the tumour-biological role of KLK6, we developed a TME model using a protease-sensitive star-shaped poly(ethylene glycol) (star-PEG)-heparin hydrogel system in which the mechanical and chemical properties are independently controlled [4].

Methodology: To mimic the extracellular components of pancreatic cancer, hydrogels were formed by covalently crosslinking protease-sensitive four-arm starPEG with maleimide-functionalized heparin. To allow integrin-mediated cell functions, hydrogels were functionalized with RGD peptides. Hydrogels were globally and locally characterized regarding their mechanical properties by shear rheometry and atomic force microscopy. To increase the complexity of our hydrogel model, and to include the cellular component of tumour tissues, human pancreatic cancer cells, together with cancer-associated fibroblasts and myeloid cells were grown encapsulated in hydrogels for 14 days. Cell viability was assessed by live/dead staining, and the metabolic activity was measured using the PrestoBlue assay. To study the role of KLK6, a CRISPR/Cas9 knockout (KO) approach has been applied.

Results: In order to mimic the stiffness of pancreatic tumour tissues, hydrogels with different mechanical properties ranging from ~4-15 kPa were achieved by varying the crosslinking degree between PEG and heparin. These results are consistent with reported data for patient-derived tissues [2] and were confirmed with our own, unpublished patient cohort. Our multicellular 3D cultures had a high viability and were metabolically active over the analysed timeframe. To test the clinical value of our TME model, the response towards treatment with different chemotherapeutics including gemcitabine and nab-paclitaxel, as well as stromal-targeting agents are assessed. Our CRISPR/Cas9 approach resulted in a successful KO of KLK6 gene expression in human pancreatic cancer cells. The functional consequences of this KO were analysed using our multicellular 3D cultures and an orthotopic xenograft approach.

Conclusion: Bioengineered starPEG-heparin hydrogels are a powerful 3D model to mimic the mechanical, chemical and multicellular characteristics of human tissues in the lab. Our future studies will now determine their potential as pre-clinical platforms for drug screening. Therefore, we are collecting patient-derived tumour specimens that will be included in our model to strengthen their clinical relevance.

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[3] Candido, J. B. et al., Cancers (Basel), 13, (2021).
[4] Mahajan, V. et al., Cancers (Basel), 13, (2021).

keywords: pancreatic cancer, starPEG-heparin hydrogels, tumour microenvironment, proteases

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AN IN VITRO VASCULARISED LIVER ORGANOTYPIC MODEL FOR THE TESTING OF NANOMEDICINES

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Nanomedicine, where therapeutic or diagnostic agents are coupled with nanoparticles (NP), offers opportunities for more efficacious treatments and accurate diagnosis of severe clinical conditions. Polymeric, lipidic, metallic or ceramic NP have been considered as carriers of these agents, but their translation into clinics requires a thorough assessment of their biocompatibility and biodistribution. However, the existing regulatory frameworks not always address the specific biocompatibility issues associated to nano-biomaterials, including cell response upon NP internalisation and accumulation within tissues. Currently available in vitro tests based on very simplistic 2D cell cultures are not suitable to mimic the clinical conditions. At the same time, in vivo animal models do not reproduce the host conditions of humans and limit the ability of tracking the NP and their interactions with cells in an accurate manner. The present paper aims to contribute to the developing of new in vitro models that can fill the gap between conventional 2D cell cultures and animal experimentation. The objective was to develop a 3D organotypic model resembling the histological features of the liver vascularised lobuli, while preserving the operator-friendly features typical of 2D cultures. Human hepatocyte carcinoma cell lines (HepG2) were co-cultured with human umbelical endothelial vascular cells (HUVEC) at a 60/40 ratio. The deriving cell suspension was seeded at a density of 105 cells/mL in a serum-free medium into tissue culture wells previously coated with a thin and completely transparent film of a synthetic biomaterial substrate, PhenoDrive-Y (Tissue Click Ltd, UK), mimicking the natural basement membrane of tissues. Lobuli-like structure formations were allowed to form over 4 days of culture in serum-free medium to limit cell proliferation, cell cycle synchronisation and cell migration. After 4 days, the cell culture was either stopped or challenged with increasing concentrations of polymeric or lipidic NP for an additional 24h. The formed lobuli-like structures were fixed in formalin and characterised for their morphology, extracellular matrix production, cytotoxicity, pro-inflammatory phenotype both in absence and presence of a challenge with NP at increasing concentrations. Uncoated tissue culture plate and Matrigel were used as controls. Time-lapse microscopy showed that, when adhering on PhenoDrive-Y, cells gradually migrated towards each other to form lobuli-like structures throughout the well surface. Confocal microscopy and scanning electron microscopy showed that angiogenic sprouting intercalated the spheroids forming each lobulus as well as covered their surface with branching. These spheroidal formations were joined together not only by angiogenic sprouting, but also by an extracellular matrix. The incubation with relatively low concentrations of either polymeric or lipidic NP (e.g. 5 micrograms/mL) showed a preferential binding of NP to the angiogenic sprouting. When challenged by increasing concentrations of NP, these lobuli-like structures appeared to lose their architecture showing clear sign of cytotoxicity.
The present work shows that 3D organotypic cultures, using liver carcinoma cells and endothelial cells and driven by a synthetic biomimetic substrate, are able to resemble the histological features of the liver vascularised lobuli thus providing valuable data of toxicity and biodistribution of NP at cellular levels.

keywords: Liver cancer, 3 D in vitro model, nanoparticles, biocompatibility, biodistribution

20941833117

A SYSTEMATIC COMPARATIVE ASSESSMENT OF THE RESPONSE OF OVARIAN CANCER CELLS TO CISPLATIN IN 3D MODELS OF VARIOUS STRUCTURAL AND BIOCHEMICAL CONFIGURATIONS

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INTRODUCTION:Termed as the ‘silent killer’,epithelial ovarian cancer (EOC) earns its nickname due to its high mortality rate, with 5-year survival rates of 46%,46.5% and 38% in UK, USA & Europe respectively.This has been attributed to some extent to the EOC’s high recurrence rate and resistance to currently available platinum based chemotherapeutic treatment methods. Hence,society, researchers and the clinical community are in dire need for a more in-depth study of the EOC microenvironment to design better patient specific treatments and to advance current therapeutic methods.Multiple groups have studied and reported the effect of chemotherapeutic agents on various EOC 3D in vitro models1,2,3. However,there are very few studies wherein a direct comparative study has been carried out between the different in vitro 3D models of EOC and the effect of chemotherapy within them.

Herein, we report for the first time, a direct comparative study of three different 3D in vitro platforms, namely (i) spheroids,(ii) synthetic hydrogels of various chemical configurations and (iii) polymeric scaffolds of various Extracellular Matrix (ECM) compositions on the cell growth and response to the chemotherapeutic(Cisplatin) for ovary derived(A2780) and metastatic(SK-OV-3) EOC cell lines.

METHODS: Spheroids of A2780 and SK-OV-3 cell lines were fabricated using specialized 96 well round bottom plates, provided by faCellitate (Manheim, Germany). Synthetic PeptiGels (Manchester BIOGEL,UK) were used as per manufacturer’s instructions for the hydrogel based culture while polyurethane (PU) scaffold assisted 3D cultures was carried out as per our previously published protocol4,5,6.Cultures were maintained and monitored for different time periods (10–28 days)depending on the culture platform type.Feasibility of using these models for assessment of chemotherapeutic agent (Cisplatin) was also carried out.Various in situ assays for monitoring the cell viability, cellular apoptosis and spatial organisation were also performed.

RESULTS: We report that all three 3D models can support the growth of EOC, but for different time periods (varying from 7 days to 4 weeks).We have seen that chemoresistance to Cisplatin, in vitro, observed especially for metastatic EOC cells, is platform dependent both in terms of structural as well as in terms of biochemical composition of the model/platform. Our study highlights the selection of the appropriate 3D in vitro model depends on the cell type,experimental time period and experimental question being asked as different models are appropriate even when studying the same disease.

CONCLUSION: We have shown the feasibility of using all three model (spheroids, hydrogels and polymeric scaffolds) for the culture of EOC cell lines and assessed the impact of chemotherapy on cell viability and apoptosis within those models. Our study highlights that the selection of a 3D in vitro platform depends on (i) the planned experimental/assessment time period, (ii) the type of cell to be studied, (iii) the site of cell origin in vivo and (iv) the question that needs to be answered.

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keywords: Epithelial ovarian cancer,Tissue Engineering, 3D in vitro model, spheroids, hydrogels, scaffolds

94238133006

HARNESSING PREDICTIVE TOXICOLOGY WITH A MINIATURIZED MODULAR GASTROINTESTINAL PLATFORM

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Introduction: The assessment of bioaccessibility and bioavailability of orally-ingested compounds is key to determine their efficacy and safety. However, there is a lack of toxicological studies that combine simulated gastrointestinal (GI) digestion with subsequent intestinal absorption. Many studies expose cells directly to pristine bioactive substances, failing to consider the series of biochemical transformations that occur throughout the GI tract. Intestinal cell lines have been widely explored to build different gut-epithelial models. However, one-fits-all approaches fall short of recreating physiologically-relevant responses. Patient-derived stem-like cells are emerging as sophisticated and refined models that ultimately surpass the potential of immortalised cells.1 Organ-on-chip offers a disruptive technology to predict in vitro toxicology, presenting a reliable approach with significant advantages when compared to in vivo and cell-based models. Here we describe a two-module microfluidic device comprising the complete GI tract, where digestive and absorptive functions are combined.

Methodology: Microfluidic digestive devices were produced from rapid prototyping (CNC micromachining) of acrylic sheets, while the cell-based module was fabricated from polydimethylsiloxane using a replica moulding technique. On-chip digestion was validated using a fluorescently-labelled casein derivative and compared to the static digestion INFOGEST protocol.2 The Gut-Chip consisted of two channels separated by an in-house fabricated membrane, coated with collagen/Matrigel. Caco-2 and HT29-MTX cells were seeded on-chip at a 9:1 ratio and cultured under continuous flow (120 µL h-1) for 7 days. Cell morphology and the epithelial barrier formation were assessed by immunocytochemistry of occludin tight junctions and by measuring the paracellular transport of Lucifer Yellow. Intestinal crypts were isolated from human colon samples of patients undergoing tumour resection surgeries, with full patient consent and approval by Ethics Committee.3 Lgr5+ cells will be expanded as organoids to be used in human primary cell organ-on-chips.

Results: Automated on-chip digestion was in agreement with the current gold standard protocol and was able to replicate typical Michaelis-Menten kinetics. Critically, our device offers enhanced time-resolution over static methods in both gastric and intestinal digestion phases, with on-line pH and temperature sensing and actuation. On-chip Caco-2/HT29-MTX co-cultures displayed 3D villi-like structures after 7 days in culture, a significantly more relevant architecture than the one obtained with Transwell inserts. Furthermore, the on-chip intestinal barrier showed a higher permeability (3.4x10-6 cm/s) when compared to the insert culture (3.0x10-7 cm/s), closely

resembling the ex vivo (4.0x10-6 cm/s). Intestinal crypts were successfully isolated from patient samples and their integration on-chip is the subject of our current goals aiming translational applications.

Conclusion: The modular microfluidic device described here shows great promise to be used as a robust tool for pharmacokinetics studies of orally-ingested compounds. The use of patient-derived organoid cultures will allow the execution of personalised studies with significant implications in both food and health applications.

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keywords: organ-on-chip, human digestion, patient-derived organoids, in vitro toxicology

94238103804

COLLAGEN-NANOCELLULOSE FORMS A MATRIX OF CONTROLLABLE STIFFNESS TO MIMIC THE PANCREATIC TUMOUR MICROENVIRONMENT

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Introduction: Bioengineered 3D cancer models allow the deconstruction of the tumour microenvironment in vitro to recreate the dynamic interactions between its extracellular and intracellular components. These bioengineered systems strongly rely on Matrigel, an undefined animal-derived matrix, to support the growth of cancer spheroids and organoids [1]. Despite its wide usage, Matrigel has poor mechanical properties and a high batch-to-batch variation, which do not capture the biomechanics of solid tumours and limit experimental reproducibility. Nanocellulose is a low-cost, sustainable and biocompatible alternative biomaterial with promising applications as hydrogel and 3D gastrointestinal organoid models [2]. In this study, collagen-nanocellulose hydrogels are used as a defined matrix of controllable stiffness to mimic elements of pancreatic tumour tissues and promote the formation of cancer spheroids.

Methodology: Nanocellulose hydrogels were synthesized by TEMPO-periodate mediated oxidation of Eucalyptus Kraft pulp suspensions and blended with bovine type I collagen solution [3]. Human pancreatic cancer cells (e.g. PANC-1, MIA PaCa-2) together with cancer-associated fibroblasts and myeloid cells were grown encapsulated in collagen-nanocellulose hydrogels for 14 days. Triple cultures were treated with the anti-cancer compound triptolide and the chemotherapeutics gemcitabine and paclitaxel. Metabolic activity and matrix stiffness were measured by Prestoblue assays and rheology.

Results: Blending of 0.2% type I collagen fibrils with 0.1% and 0.2% cellulose nanofibres formed a matrix of controllable stiffness, with a Young's modulus ranging from 647 ± 69 to 1,189 ± 234 Pa. Pancreatic cancer cells formed spheroids of 90 ± 30 µm diameter. Cell-containing matrices reached a Young's modulus of 3,303 ± 226 Pa, which resembles the lower profile of pancreatic cancer tissues [4]. Treatment with triptolide, gemcitabine and paclitaxel reduced the cell viability of triple cultures by 45 ± 2%. The exposure to all three drugs combined reduced the Young's modulus of the MIA PaCa-2 triple cultures by 42 ± 2%, whereas the stiffness of those containing PANC-1 cells decreased only by 8 ± 3%

Conclusion: The mechanical properties of collagen-nanocellulose matrices are controlled by varying the concentration of cellulose nanofibres. The incorporation of pancreatic cancer and stromal cells into the biomimetic hydrogels demonstrates the importance of the cellular elements for matrix stiffening. Drug treatments modulate the mechanical properties of this 3D cancer model, resulting in differential cell responses. Collagen-nanocellulose stands as an alternative matrix to Matrigel to recreate the tumour microenvironment and support the growth of cancer spheroids, as well as to screen novel or improved treatments.

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keywords: hydrogel, tumour microenvironment, pancreatic cancer, 3D model

S02
3D in vitro tissue-engineered cancer/disease models
Session II - Room: S1
(28 Jun 2022, 13:30 - 15:00)

Conveners: Serena Danti; Rui L. Reis

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MIMICKING THE TUMOR STROMA-INDUCED VASCULATURE COLLAPSE IN 3D PANCREATIC TUMOR MODEL

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is one of the most fibrotic tumors, which can possess up to 90% tumor stroma of the total tumor mass (1). The tumor stroma is comprised of cancer-associated fibroblasts (CAFs), extracellular matrix (ECM) and many immune cells. The physical and biochemical characteristics of the tumor stroma control cancer cell proliferation, invasion, and metastasis. Also, the tumor stroma enhances intratumoral solid stress leading to the compression and collapse of blood vessels and also becomes a physical barrier which in turn prevents delivery of chemotherapy. Currently, there is lack of relevant in vitro models that are able to replicate these mechanical characteristics of PDAC (2).

Methodology: A multi-layered vascularized 3D PDAC model was developed consisting of primary human pancreatic stellate cells (PSC) in a collagen/fibrinogen (Col/Fib) matrix. A central endothelialized channel was introduced to study the cell-mediated contraction of vasculature in PDAC. The activation of PSCs due to mechanical and biological stimulation was studied using contraction assay and gene analysis. The clinical relevance of the model was studied for PDAC specific gene markers and compared with publicly available patient data. The effects on the blood flow inside the channel was determined using computational fluid dynamics (CFD) simulations. The effect of AV3 peptide (3), an integrin inhibitor developed by us, was tested for its effect on stroma and vasculature in this model and the effects were correlated with in vivo data in mice.

Results: PDAC samples from patients and mouse tumor models showed an abundant stroma and collapsed blood vessels. Our 3D model showed mimicking of the vasculature in the tumor tissue and adjunct healthy tissue. PSCs differentiated into myofibroblastic CAFs leading to high contraction of the matrix and upregulation of gene markers (ACTA2, Col-1a1, PDGFbR, HAS2 and CDC42). Interestingly, these genes were also positively correlated in PDAC clinical samples. The CFD simulation analysis revealed a clear pressure drop within the compressed vessels with high intravascular pressure before the compression. Furthermore, the flow velocity drastically increased in the compressed vessels. Interestingly, treatment with AV3 suppressed the compression of vessels which according to CFD simulation should result into enhanced drug delivery. The latter was proven in stroma-rich PANC-1+PSC and MiaPaCa+PSC tumor models in mice. We found that treatment with AV3 reduced desmoplasia, decompressed vasculature and enhanced delivery of chemotherapy in vivo.

Conclusion: Altogether, our 3D PDAC model provides a better understanding of mechanical characteristics of PDAC in view of stroma-induced vasculature compression as well as allows for evaluating novel anti-stromal therapeutics for the treatment of fibrotic tumors.

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keywords: Tumor stroma, pancreatic tumor, 3D in vitro model, vasculature, fibrosis, tumor microenvironment

73296306486

BOTTOMS-UP BIO-PRINTING OF CELLULARIZED POROUS MICRO-SCAFFOLDS TO ENHANCE CELL PROLIFERATION, VIABILITY AND MIGRATION

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Extrusion bio-printing is the most direct and inexpensive method for printing three-dimensional cell models. This technique provides interesting solutions to generate more complex architectures than the already existing 3D models but still presents significant drawbacks that once solved will improve its field of application in regenerative medicine and advanced 3D biological models. To print complex structures with a larger volume, it is necessary to have available an important quantity of cells. There is also significant cellular stress during the printing process when extruding the ink, due in part to shear stress, which can induce the apoptosis or inability of the deposited cells.1 One of the common answers to these problems is the use of soft hydrogels having little mechanical strength, implying a lack of structural integrity of the printed designs. For this purpose, we produced new hyper porous micro-scaffolds of PLGA reducing the shear stress experienced by printed cells.

We developed a novel method of producing porous PLGA with a simple double emulsion and enhanced the cell adhesion of the particles' surface by adding two types of coating. With these micro-scaffolds, we put in place a 3D cell culture method using said micro-scaffolds to improve cell proliferation before printing by acting as micro-carriers. We combined these cellularized micro-scaffolds with a bottom-up method of bio-printing of complex 3D structures and integrated them into various types of bio-inks, engineered either for the maximum survival of cells or for the construction of highly complex 3D structures. Ultimately, these micro-scaffolds are capable of protecting the cells during the bio-printing process by absorbing most of the shear stress inherent to all extrusion bio-printing. We also printed more complex structures, composed of structured layers of stained mesenchymal and cancerous cells, thus creating an organoid. The observation in time of the movement of cells inside the organoid allowed us to quantify the interaction and migration of cells with and without our micro-scaffolds.

We produced Polylysine or Collagen coated PLGA micro-scaffolds with an average size of 100 µm in diameter with a porosity of 25-45 %. Our results show an augmentation up to 400% of cell proliferation when cultured with said particles. The viability after printing is augmented with the use of our micro-particles, with a survival rate between 85 and 91% with our particles and between 75 and 83% without the micro-scaffolds. When printing more complex structures with co-cultures of tumorous cells with mesenchymal cells, the presence of our micro-scaffolds increase the migration of stem cells towards the tumorous cells.

Finally, the overall results offer new insights regarding bio-printing and cellular proliferation and migration in response to the presence of micro-scaffolds. Our new process promotes high cell productivity and viability before and during bio-printing. The use of our micro-scaffolds would make it easier, faster and more efficient to produce three-dimensional cellular structures and to analyze the behaviour and interactions of different cell types in this 3D environment.

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keywords: Bio-printing; PLGA micro-scaffolds; organoid

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POST-PRINTING STRUCTURE FORMATION IN BIOPRINTED TISSUE CONSTRUCTS THAT MIMIC THE TUMOR MICROENVIRONMENT

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Understanding the key players in cancer progression is essential for the development of effective therapies. Aiming to pinpoint the roles of biochemical and biophysical factors involved in malignancy, tissue engineers developed in vitro cancer models of increasing complexity [1]. Three-dimensional (3D) bioprinting techniques were extensively used in this endeavor [2,3] due to their ability to create biomimetic spatial patterning of several cell types that coexist with cancer cells in the tumor microenvironment (TME), including tumor-associated fibroblasts, immune cells, mesenchymal stem cells, adipocytes, and vascular cells [4]. Nevertheless, bioprinted cells rarely remain where the bioprinter delivers them; they remodel their extracellular matrix and take advantage of their motility to establish firm bonds with other cells and/or biomaterials [5]. Therefore, in the present work, we investigated structure formation in bioprinted models of the TME both experimentally and computationally [6]. We used extrusion-based bioprinters to build models of the TME. SK-BR-3 breast cancer cells dispersed in a hydrogel droplet (106 cells/mL) were embedded in the same hydrogel (Bioink, CELLINK, Sweeden) loaded with tumor associated fibroblasts (TAFs − 5×105 cells/mL) and peripheral blood mononuclear cells (PBMCs − 5×105 cells/mL) harvested from breast cancer patients. The bioprinted tissue constructs were cultured in vitro for two weeks and cryosectioned for histological evaluation. Hoechst staining demonstrated that cells remained viable and remodeled the hydrogel. Hematoxylin and eosin (H&E) staining of histological sections, prepared at various time points, indicated that cells proliferated and formed heterotypic aggregates of malignant and peritumoral cells. To investigate the interactions responsible for the observed phenomena, we built lattice models of the bioprinted constructs and simulated their evolution using Metropolis Monte Carlo methods [6]. The computational model was formulated on a 3D cubic lattice, representing the biological system, at single-cell resolution, in terms of 4 types of particles: tumor cells, peritumoral cells, volume elements of the hydrogel, and volume elements of the cell culture medium. Based on the differential adhesion hypothesis, computer simulations reproduced most features of the experimentally observed structure formation, but did not account for the superficial localization of the heterotypic aggregates. Depending on model parameters, peritumoral cells wrapped or infiltrated cancer cell aggregates, as expected from TAFs and immune cells, respectively. Despite their limited complexity, the tissue constructs developed in this study could be used to establish co-culture conditions for cancer cells, TAFs, and PBMCs. Future investigations should consider model tissues incorporating perfusable channels with endothelial cell lining. Also, the computational model needs to be extended to describe the self-assembly of different cell types present in the native TME.
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keywords: extrusion bioprinting, breast cancer models, tumor associated fibroblasts, peripheral blood mononuclear cells, Monte Carlo simulations

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BIOREACTOR DYNAMIC ORGANOTYPIC CULTURE OF PRIMARY LIVER CANCER AS A PERSONALISED IMMUNOCOMPETENT DRUG SCREENING PLATFORM FOR IMMUNO-ONCOLOGY

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Introduction: Current primary liver cancer models fail to truly encompass the human tumour immune microenvironment, exacerbating a recognised discord between the preclinical and clinical successes of emerging (immuno)therapeutics. The organotypic 3D culture of human precision-cut tumour slice (PCTS) is a cancer explant model which retains tumour specific histoarchitecture, aetiological background, disease phenotype, resident immune landscape, and checkpoint expression for up to 7 days ex-vivo. Our study aims to advance culture conditions of PCTS using a proprietary Multi-well Plate (MuPL) perfusion bioreactor to extend culture lifetime and allow perfusion of immune cells through PCTS, in order to validate PCTS as a tool to assess patient-specific therapeutic responses.
Methodology: PCTS generated from primary liver cancer (hepatocellular carcinoma and cholangiocarcinoma) were treated with approved single agent or combinatory checkpoint inhibitor monoclonal antibody (CPI-mAb) or kinase inhibitor (KI) therapy for up to 7 days. PCTS/immune cells co-cultures in the MuPL bioreactor were longitudinally assessed for viability, histology, and tissue integrity. Therapeutic response was determined by evaluating histology (H&E and Sirius red), apoptosis/cell death (TUNEL, lactate-dehydrogenase release, cytokeratin 18), and proliferative capacity (PC; Ki67). Gene expression was assessed using QuantiGene RNA Assay. Resident immune cells were assessed by immunofluorescence and FACS.
Results: PCTS and immune cells co-cultured in the MuPL bioreactor maintained viability, structural integrity and histoarchitecture for >7 days. Doxorubicin was used as a positive cell death control in all treated patients, decreasing PCTS viability and PC by day 5. Compared to monotherapy, nivolumab (CPI-mAb) + regorafenib (KI) therapy decreased the tumour-to-stroma ratio and PC in all patients by day 7. Also, significantly increased apoptosis was detected in one patient, who comparatively showed higher checkpoint expression including PD-1, PD-L1 and CTLA-4. Other combinatorial immunotherapies, including atezolizumab + bevacizumab and nivolumab + ipilimumab (CPI-mAbs), reduced PC without affecting histology or viability. The overall immunotherapeutic response was patient-specific.
Conclusion: PCTS can be used as a powerful tool to study personalised responses to (immune) therapeutics. In addition, PCTS can be successfully cultured in our proprietary perfusion system, recapitulating tumour-immune cell interactions, allowing assessment of response to cell and

vaccine therapy ex-vivo.

keywords: liver cancer, bioreactor, immunotherapy, liver slices

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DEVELOPMENT OF A BIOPRINTED BREAST CANCER MODEL USING DECELLULARIZED MAMMARY GLANDS

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Introduction
3D bioprinting has emerged as a promising technology for fabricating artificial tumors as it allows the fabrication of complex models recreating tumor physiology. The importance of the extracellular matrix (ECM) in tumor progression, cancer cells and stromal cells crosstalk and drug resistances, has motivated the development of more biomimetic tumor-ECM bioinks that recapitulate the high complexity of the ECM.1 In this regard, decellularized tissues-derived matrices (TDMs) can provide the native tissue biological cues, but its inadequate mechanical properties prevent their bioprinting. The aim of this work is to develop a breast TDM-like bioink suitable for bioprinting breast cancer models without the need of a sacrificial material.
Methodology
Porcine breast tissues were decellularized and delipidated, and its composition was studied. TDMs pre-gels were fabricated by digesting it with pepsin and neutralizing the pH. The addition of rheological modifiers into the bioink was also assayed. TDM bioinks were printed with a 3D bioplotter (RegenHU) and then crosslinked. The bioinks were further tuned by incorporating an ECM protein overexpressed in breast cancer, Collagen type 1 (Col1). The shape fidelity, printability and rheological properties of the bioinks were characterized and the hydrogels Young modulus was measured. For bioprinting artificial breast tumors, cell-laden bioinks were prepared by dispersing breast cancer cells (BCCs) or mesenchymal stem cells in the bioink. Cellular survival, proliferation, morphology, and the expression of adhesion molecules were studied. The bioprinted hydrogels were used to study the efficacy of anticancer drugs.
Results
Breast TDMs were successfully decellularized and rich in glycosaminoglycans and collagen. The addition of rheological modifiers allowed the TDM bioprinting without the requirement of any sacrificial material. BCCs were able to proliferate in TDM bioprinted scaffolds and form spheroids with a low expression of e-cadherin. The addition of Col1 improved the bioink printability, increases cellular proliferation and reduces doxorubicin sensitivity. TDM bioinks also allowed BCCs and stromal cells bioprinting and therefore could be used to fabricate artificial tumors.
Conclusions
Taken together, we have proved that TDM bioinks could be used for bioprinting artificial breast tumors closely recreating the tumor ECM.
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keywords: 3D printing, mammary gland, decellularization, 3D cancer modeling

31412737539

INVESTIGATION OF BREAST CANCER EPITHELIAL-MESENCHYMAL TRANSITION USING 3D COLLAGEN-BASED MODELS

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Treatment options for triple-negative breast cancer (TNBC) are limited. Current 2D cancer models fail to accurately model the tumour microenvironment of breast cancer. Alterations to extracellular matrix (ECM) composition have been shown to play a key role in the epithelial-mesenchymal transition (EMT) process involved in breast cancer progression (1). This highlights the need for the development of a representative in vitro 3D model, in which to study TNBC behaviour and identify new treatment targets. This project aims to develop a collagen-based scaffold model composed of matrix components of breast tissue, including glycosaminoglycans, hyaluronic acid (HyA) and chondroitin sulphate (CS), both elevated in tumours, to investigate their role in the EMT process, within TNBC.

Collagen-based scaffolds comprised of varying concentrations of HyA, or CS were fabricated using previously optimised protocols (2). Scaffold characterisation was performed using SEM, porosity measurements, and mechanical testing. AlamarBlue and DNA assays were performed to assess the metabolic activity and growth of TNBC cell lines, MDA-MB-231 and MDA-MB-436, in comparison to normal breast epithelial cell line, MCF10a. The migratory ability of TNBC cells in 3D was assessed with H&E staining. A cytokine array and qPCR were performed to assess the effect of varying HyA and CS concentrations on MCF10a and MDA-MB-231 behaviour and to determine expression levels of markers associated with the EMT process.

All scaffolds were highly porous and had a uniform pore distribution, with an average stiffness of 1kPa and are therefore within the stiffness range of cancerous breast tissue (1kPa-4kPa). Each scaffold type exhibited huge biocompatibility for each cell line. TNBC cells were more metabolically active on CHyA scaffolds than CCS scaffolds and TNBC cells proliferated at a faster rate than MCF10a cells. Each scaffold type supported the migration of TNBC cells. Change in HyA or CS concentration did not affect cell proliferation but altered the expression of pro-inflammatory cytokines. Interestingly, an increase in HyA increased pro-inflammatory cytokine expression and an increase in CS decreased pro-inflammatory cytokine expression in MDA-MB-231 cells. The effect of glycosaminoglycan type and concentration on cytokine expression requires further investigation. Alterations in the expression of EMT associated markers differed with an increase in HyA and an increase in CS concentration, findings which require further investigation.

Collagen-based scaffolds composed of varying ECM components have been developed. Altering the mechanical stiffness of the collagen-based scaffolds within a range that represents cancerous breast tissue is achievable. Each scaffold is highly porous and supports cell viability and proliferation. Varying concentrations of HyA and CS alters the cytokine expression profile of MCF10a and MDA-MB-231 cells, highlighting the effect of changes to the ECM composition on cancer progression. This finding will be further investigated in future studies. In summary,

the collagen-based scaffolds have the potential to mimic the ECM of breast tissue and have the capacity to be used as 3D models for breast cancer research.

Acknowledgements: Funded by Health Research Board

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keywords: Breast cancer, 3D collagen-based scaffolds, Epithelial-Mesenchymal Transition

20941831204

ENGINEERING BIOMIMETIC HUMAN LUNG TUMOR MODELS

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Introduction
Lung cancer is the leading cause of cancer mortality with poor prognosis due to late stage diagnosis, drug resistance and high risk of relapse. There is a high need for tissue engineered 3D models that can recapitulate tumor heterogeneity and complexity to understand the cellular mechanisms leading to lung tumorigenesis, metastasis and drug responses. Future of precision oncology is envisioned with the ability to perform therapeutic tests on pre-clinical tumor models that can faithfully recapitulate the native tumor microenvironment combined with the use of patient-derived cells that would inform the treatment decision making. Patient-derived xenografts (PDX) and tumor-derived organoids (TDO) have emerged as methods to provide reliable pre-clinical models that account for genomic diversity and cellular heterogeneity especially for cancers with lack of established cell lines. However, PDX models are costly with low tumor formation rate that limit medium to high-throughput screening approaches. On the other hand, TDO allowed in vitro culturing of both solid tumor and liquid biopsy-derived cells in Matrigel and revealed preservation of genomic diversity. Nevertheless, Matrigel culturing hinders a systematic study of the role of tumor extracellular matrix (ECM) components and mechanics on tumor cell growth, phenotype, metastatic potential and drug responses.

Methodology
Lung tumors are marked by an increase in tissue stiffness as well as changes in the biochemical composition (i.e. increase in cell-instructive ECM ligands). We developed a biomaterial-based human in vitro human non-small cell lung adenocarcinoma model to study the effect of aberrant tumor matrix characteristics in a controlled manner on the phenotype and malignancy of pulmonary epithelium. We built models for healthy and tumorous lung matrix from hydrogels of decellularized native lung extracellular matrix (ECM) with differing ligand content and tissue stiffness. We then encapsulated non-small cell lung adenocarcinoma cells (A549) in both healthy-like (low stiffness, low ligand content) and tumor-mimetic lung matrices (high stiffness, high ligand content) and monitored cell growth and phenotype over 4 weeks. We performed analyses on gene (qRT-PCR, RNAseq) and protein expression (Western blot, immunofluorescence) to investigate the signaling mechanisms involved in the tumor matrix-mediated effects on cell growth and phenotype. We performed loss of function (small molecule, shRNA) and overexpression studies to validate proposed mechanisms triggered in the cells in the respective engineered microenvironments.

Results
Lung tumor cells in tumor-mimetic matrices demonstrated significantly higher cell growth with increased number of larger and invasive-looking (decreased circularity) colonies. Gene and protein expression analyses revealed an upregulation of the epithelial-mesenchymal transition program with significantly higher expression of known markers including N-Cadherin, Zeb-1 and Twist-1 as well as lung adenocarcinoma markers such as TTF-1 when compared to heathy-like microenvironments. Mechanistic studies revealed ECM-ligand mediated induction of aberrant growth and tumorigenic phenotype that synergizes with the increased mechanical stiffness in the microenvironment.

Conclusions

Understanding the key characteristics of lung tumor microenvironment and recapitulating the compositional and mechanical differences in tissue engineered models hold a great importance towards achieving cellular responses seen in patients to steer therapeutic approaches for better clinical outcome.

keywords: cancer tissue engineering, lung tumors, extracellular matrix, biomimicking

83767212955

THE BIOMECHANICAL SIGNATURES OF 3D IN VITRO TUMOUR MODELS

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INTRODUCTION: Most epithelial cancer cell populations undergo an epithelial to mesenchymal transition (EMT), acquiring a more aggressive phenotype1. Mesenchymal cells are more motile and have the ability to remodel the extracellular matrix. This mechanical interaction with the surrounding matrix can be measured by bulk and single cell force generation. In this study we aim to use 3D in vitro methods to assess the contractility signatures of various epithelial cancer cell-lines.

METHODS:
Contraction assays. Two colorectal cancer (HT-29, HCT 116) and two breast cancer (MDA-MB-231, MCF-7) cell-lines were incorporated into collagen type I hydrogels at 1x106 cells/mL. Contraction was observed over 96 hours. Human Dermal Fibroblasts (HDFs) serve as a positive control.

Traction force microscopy (TFM). Polyacrylamide (PA) gels containing 0.1 μm red fluorescent beads were cast on glass bottom petri dishes. The gels were functionalised using collagen type I. Cells were seeded on the gels at a density of 1.5x105 cells and incubated for 24 hours before imaging. Comparative images were taken before and 15 minutes after trypsinization of the cells.

Quantitative polymerase chain reaction (qPCR). RNA was extracted from hydrogels through the TRI- Reagent® phase-separation method2. The following genes were measured for relative expression: LOX and RAE1.

Analysis. Images were analysed using Fiji ImageJ software and statistical analysis were performed using GraphPad Prism 9.

RESULTS: HDF cells contract the collagen type I hydrogels by 60% over 24 hours. MDA-MB-231 cells cause a 22% contraction whilst HT-29, HCT 116 and MCF-7 cells did not contract the gel. TFM results validate this trend showing that HDF cells displace the matrix by 2.8±1.7 μm. MDA-MB-231 cells displace the matrix by 0.7±0.1 μm. HCT 116, HT-29 and MCF-7 cells do not displace the matrix. MDA-MB-231 cells has significant upregulation of the EMT markers LOX and RAE1 compared to HT-29 cells ($p=0.0070$ and $p=0.0237$ respectively).

CONCLUSION: This study showed a correlation between the contractility profiles of epithelial cancer cells and their EMT status: highly mesenchymal-like cancer cells such as MDA-MB-231 cells are force-generating cells. This suggests that these cells can remodel the extracellular matrix, which aids migration and hence makes them highly invasive cancer cells.

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keywords: cancer, EMT, forces, extracellular matrix, 3D models

S03+S33
3D printing of bionic organs – how far are we from clinical application? + From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine
Room: S3 B
(28 Jun 2022, 15:30 - 17:00)

Conveners: Marta Klak; Jakub Rybka; Lukasz Witek; James E. Smay

20967801564

3D-BIOPRINTED BIONIC PANCREAS AS AN INNOVATIVE METHOD OF TREATING AND PREVENTING DIABETES – HOW FAR WE ARE FROM CLINICAL APLICATION?

brak autora

Introduction

Type 1 diabetes (T1D) is a disease, which affects milions of patients. Islet or pancreatic transplantation is a method of treating complicated T1D. The limitation of these methods is the lack of organs for transplantation. 3D-bioprinting using living cells could be a solution. We present results of bioprinted bionic pancreas on mouse and pig model.

Materials and Methods

Research was carried out on 60 mice (SCID) and 24 pigs. The mice were divided into 3 groups: control; IsletTx in which porcine pancreatic islets were transplanted under the renal capsule; 3D-bioprint in which bioink petals consisted of bioink A and porcine islets. The bioprinted petals were transplanted into the dorsal part of the muscles under the skin in mice. Daily glucose measurement was performed and the level of C-peptide was tested every 7-days.
The pigs were divided into 4 groups: control, diabetic group(pancreatectomy-T1D); with transplanted 3D-bioprinted bionic petals(TX-with previous pancreatectomy), pigs with transplanted 3D-bioprinted bionic organ with full vasculature. The animals were measured daily with blood glucose levels (from 5-20 measurements per day). 3D-bioprinted bionic pancreas were transplanted in some animals to the iliac vessels and in other subgroup to the aorta and vena cava.

Results and Discussion

The results obtained in mice initially showed no differences in the concentration of peptide-C and glucose between groups. However, as early as 7-days after transplantation, both parameters analyzed in the fasting state were significantly lower in the IsletsTx and 3Dbioprinted groups compared to the control group. On day 14, decreased values of C-peptide and glucose were observed only in the group with petals transplants.
Mean glucose levels were two times lower, compared to the period before petals transplantation. In addition, TX pigs required lower doses of insulin after petals implantation. After transplantation of 3D-bioprinted bionic pancreas, a stable flow through the organ was observed in vivo and after the excision of the organ.

Conclusions

Transplantation of bionic petals in mice and pigs resulted in a decrease in mean glucose levels. None of the animals died due to postoperative complications or the lack of biocompatibility with the bionic structure. Transplantation of fully vascularized organ created with 3D-bioprinting technology id feasible.

Acknowledgments

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Accomplished by the Bionic Consortium - Foundation of Research and Science Development,

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keywords: T1D, Pancreas, bioink, islets, 3D Bioprinting

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TISSUE ENGINEERED SCAFFOLDS FOR TRACHEAL REGENERATION: A SEEDING APPROACH IN A MULTI-LAYERED 3D PRINTED SCAFFOLD

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Tracheal damage is associated with the narrowing, weakening and discontinuity of the conductive part of the lower respiratory tract. Extensive defects cannot undergo end-to-end anastomosis and current approaches present poor outcomes due to weak mechanical properties, poor re-epithelialisation and vascularisation of the implanted graft. Herein, we investigated the use of collagen-based tubular scaffolds reinforced with 3D-printed synthetic polymer architectures for tracheal repair. A collagen and hyaluronic acid film covered the inner lumen (IL) of the scaffold, using CHyA-B scaffolds to support the formation of a respiratory epithelium. The 3D printed reinforced collagen porous outer layer (OL) of the scaffold was designed to support the growth of underlying tissues including cartilage and connective tissues as well as the formation of a vasculature network around the graft. The mechanical strength and ultrastructure of the tubular scaffolds was characterised and an approach for cellular seeding of the different layers of the scaffold was developed.
Tracheal scaffolds showed a 10-20 compressive MPa Young modulus with no significant decrease in mechanical strength following cyclic loading used to mimic respiratory patterns. Scaffold characterisation revealed a porous microarchitecture using scanning electron microscopy imaging with mean pore size of 169.7±11.2 µm suitable for cellular proliferation estimated via toluidine staining. A seeding process with the support of a custom-made device and 3D printed accessory parts was developed to achieve targeted epithelial seeding (Calu-3 bronchoepithelial cells) on the IL while the outer layer OL of the scaffold was populated with Wi38 lung-derived fibroblasts using different seeding densities under rotation. The growth of Wi38 cells on the OL was monitored for 7 days, showing successful cellular growth on the OL with no cellular attachment and growth in the IL using 6x105cells/cm2. Calu-3 cells were grown on the tubular scaffolds for 10 days, showing optimal cellular growth on the IL of the scaffold using 1.25x105 cells/cm2 with little attachment and growth of Calu-3 cells in the porous OL. Immunofluorescence imaging and quantification of the film from the IL further demonstrated cellular growth on the film with an estimated epithelial coverage of the film >60%.
Reinforcement of CHyA-B scaffolds with 3D-printed polymer architectures represents a suitable approach for the development of tissue-engineered tracheal grafts, showing adequate mechanical properties and an optimal porous structure to support the formation and growth of tracheal tissues. Moreover, a seeding procedure using a custom-made device was developed allowing successful cellular attachment and growth in the different layers of the tubular scaffold. The establishment of this targeted cellular seeding procedure holds potential to enable the clinical translation of tissue engineered tracheal grafts by facilitating differentiated pre-seeding strategies prior to implantation.

keywords: trachea, collagen, 3D printing, epithelialisation, tubular.

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BONE REGENERATIVE CAPACITY OF 3D PRINTED BIOACTIVE CERAMIC SCAFFOLDS COATED WITH BIOACTIVE MOLECULE: DIPYRIDAMOLE

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Extensive defects of the upper extremity cause significant patient burden, including disability and social stigma. Approximately, 500,000 bone defects are reconstructed annually in the USA alone at a cost of ~.5 billion, due to factors including donor site harvest and lengthy operative times. Bone defects >5cm are usually reconstructed with autologous vascularized bone transfer (bone from another region of the patient's body is harvested to replace the defect), but limitations include donor site morbidity, infection, and delayed healing. These limitations drive innovation of biomaterial applications, but a tissue engineered approach to reconstruction remains elusive. The objective of this study was to assess the efficacy of 3D printed bioactive ceramic (3DPBC) scaffolds augmented with dipyridamole (DIPY), an indirect A2AR agonist known to enhance bone formation, to stimulate bone regeneration of a critical-sized defect of the radius in an in vivo translational model. A 3DPBC scaffold was utilized to repair critical sized long bone defects in-vivo. In this study, 3DPBC scaffolds were fabricated in a two-piece system. Following IACUC approval critical-sized full thickness (~7xm x full thickness) defects were created in the tibia diaphysis in sheep (N=8). The 3DPBC scaffold composed of β tri-calcium phosphate (β-TCP) was placed into the defect site, along with an intramedullary rod and animals were euthanized 24 weeks. The tibia were retrieved, for micro-CT, histological and mechanical analysis. Bone growth was assessed exclusively within scaffold pores and evaluated by microCT and advanced reconstruction software. Biomechanical properties were evaluated utilizing nanoindentation to assess the newly regenerated bone for elastic modulus (E) and hardness (H). Qualitative evaluation of the histological micrographs indicated directional bone ingrowth of bone, with an increase in bone formation toward the native bone morphology. Extensive bone formation with signs that scaffold has significantly resorbed, presenting areas of extensive structural discontinuity resorption was observed at both low and high magnifications. Histological micrographs at high magnification to better appreciate the features of the newly regenerated bone within the scaffold. Furthermore, qualitative evaluation did not yield any exuberant bone growth and the newly regenerated bone was limited to the defect and the scaffold regions. Our previous studies using a smaller preclinical model, rabbit, yielded favorable results, where with the implantation of a custom-fit 3D printed resorbable bioactive ceramic scaffolds into critical size radius defects resulted in bone morphology that remarkably resembled the original bone segment with a haversian cortical shell presenting cortical-like mechanical properties and associated marrow space. The application of this β-TCP scaffold has the potential for successful treatment outcomes while potentially minimizing the amount of surgery and less time spent in hospitals for individuals that would not fully recover

from injury though current technology and treatment strategies available. Custom engineered, biocompatible and resorbable, β-TCP scaffolds treated with DIPY demonstrated to have an increased bone regeneration qualitatively and quantitatively. The custom approach and expedited healing has the potential to positively benefit the patient in terms of lowering health care costs and patient's quality of life, as well as returning to form and function.

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keywords: 3D printing, Scaffolds, In vivo, bone, tissue engineering

94238123688

SCAFFOLD GUIDED BONE TISSUE ENGINEERING FOR THE ASSESSMENT OF BONE DEFECT RECONSTRUCTION – PRE-CLINICAL AND CLINICAL TRIALS

Flavia Medeiros Savi (Queensland University of Technology, Brisbane, Australia), Dietmar W. Hutmacher (Queensland University of Technology, Brisbane, Australia)

Introduction. Irrespective of the several scaffold designs that have been investigated in the last 30 years, the actual number of scaffold guided bone tissue engineering (SGBTE) approaches that were able to reach clinical application are few. Most of these approaches fail translation into clinical settings firstly because outcomes of scaffold design properties and host immune responses results are poorly correlated, and secondly because accurate prediction on how they will behave in humans are mostly based on lower levels of organization animal model findings. To develop a de novo understanding of the biocompatible mechanisms of SGBTE processes upon implantation of 3D printed medical grade Polycaprolactone (mPCL) scaffolds, and the prospective of exploiting novel concepts and material design innovation, requires a rigorous pre-clinical experimental demonstration of therapeutic promise in clinically relevant animal models. Over the last twelve years, our research group has trialled a number of SGBTE concepts using our established sheep animal model as a pre-clinical tool for evaluating bone tissue reconstruction. Here we provide an overview of the pre-clinical segmental bone defect studies performed by our group in the last twelve years, as well as an overview of the SGBTE concepts that were able to reach clinical applications. Methodology. Studies used 3D printed mPCL scaffolds (Osteopore International, Singapore) in combination with a variety of mediators, including autologous bone grafts, autologous and allogenic mesenchymal bone marrow precursor, platelet rich plasma, and bone morphogenic proteins. Scaffold mechanical properties have been assessed. Merino sheep aged ≥ 6years old was the animal model used for all studies. Conventional X-rays, ex-vivo biomechanical testing, Micro computed tomography (µCT), histology, immunohistochemistry, scanning electron microscopy, and histomorphometry were used to monitor healing progression. Results. A total of eighteen pre-clinical and seven clinical studies were performed in the last twelve years. All animals recovered from surgical interventions and completed the experimental period uneventfully. Using state-of-art µCT, histological, immunohistochemical, image analysis techniques and innovative quantitative analysis, these studies have led to significant understanding of the bone biology, on the biocompatible mechanisms of SGBTE during the regeneration processes, as well as, on providing new insights into mimicking the natural bone tissue regeneration environment in large animal models. These studies were paramount in the development of a pre-clinical model protocol for assessing bone regeneration in large bone defects, instrumental on the world-first patient and largest segmental bone defect to be successfully reconstructed using a mPCL scaffold (not published) and on a femoral shaft critical-sized bone defect reconstruction.
Conclusion. While a lot of effort has been invested in optimization scaffold parameters, currently, there is a growing interest with much of the focus on profiling large animal model's bone responses to 3D printed medical devices, with further emerging evidence suggesting that the scaffold architecture is a niche where adaptative immune cells are decoding scaffold features. As such, the in vivo evaluation of the bone responses through pre-clinical large animal models is an unavoidable component of translational research and should be used to justify and establish scaffold guided tissue engineering concepts in clinical settings.

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keywords: scaffold guided bone tissue engineering, critical-sized bone defects, FDA-approved, large animal models

83767212328

SELF-ASSEMBLING PEPTIDE HYDROGELS AS BIOINKS FOR 3D BIOPRINTING APPLICATIONS

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INTRODUCTION

With a growing demand for effective regenerative medicine therapies, more sophisticated tissue-engineered in vitro models are required for a better understanding of the fundamental biological processes that underlie regeneration. To tackle this need and further comprehend these processes, new technologies are emerging in the tissue-engineering field. The state-of-the-art technology of 3D bioprinting aims to achieve well-defined biological structures by printing cell- embedded hydrogels or bioinks in a layer-by-layer manner. A main challenge of 3D bioprinting is the lack of "soft" bioinks with a wide printability window, which offer adequate biofabrication properties as well as a cell-friendly extracellular matrix (ECM)-like microenvironment. Thus, allowing the encapsulation and culture of cells in complex in-vitro 3D tissue models1.

Self-assembling peptide hydrogels (SAPHs) are fully defined, semi-synthetic hydrogels, which are biocompatible and with tuneable mechanical properties. Therefore, SAPHs are believed to stand as a powerful option with unique properties that make them perfect candidates for this purpose2. Herein, this research aims to design and explore SAPHs as novel bioinks for extrusion-based 3D bioprinting.

MATERIALS AND RESULTS

To characterise subject hydrogels, rheological analyses, printability and cytocompatibility tests were carried out using oscillatory rheology, extrusion-based bioprinting and human Mesenchymal Stem Cells (hMSCs), respectively. Rheological analyses showed that our subject peptide-based hydrogels were shear thinning and recovered well under shear stress. Relaxation times fitting curves revealed the characteristic dynamic times in which our hydrogels recovered following a classical mechanical model3. All these rheological findings related to good printability in shape fidelity and integrity analyses. We investigated fibroblast and hMSC viability to assess the biocompatibility of the hydrogels. These studies resulted in SAPHs being promising printable and biocompatible biomaterials for extrusion-based 3D bioprinting with good biofabrication attributes.

CONCLUSION

We have successfully developed and tested SAPHs as bioinks and assessed cell viability over a 21-day culture period of bioprinted embedded-fibroblast and hMSC hydrogels. An application we are currently exploring, is to investigate if bone differentiation could be induced to determine how capable these constructs are to differentiate into physiologic bone phenotype4. Translated to real-world use, the biofabrication of bone and cartilage models through 3D bioprinting could result as a powerful tool for in vitro disease modelling and to treat bone conditions as osteoarthritis in early stages of the disease.

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keywords: 3D Bioprinting, Self-assembling Peptide Hydrogels, Bioinks, Mesenchymal Stem Cells

94238135204

BIODEGRADABLE AND BIOACTIVE PERSONALIZED IMPLANT FOR GUIDED BONE REGENERATION

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INTRODUCTION

One of the main functions of guided bone regeneration (GBR) barriers are to preserve the bone graft and maintain its mechanical stability during the healing process. Personalized metallic meshes meet GBR demands as well as a good predictable tissue regeneration1. Although they offer good performance in terms of tissue regeneration, these metallic meshes present several drawbacks such as a second surgery to extract the mesh, autologous bone extraction from other anatomical locations, or mesh exposition. These problems cause extra pain and morbidity to the patient. Therefore, this project aims to substitute the use of metallic meshes with patient-specific biodegradable implants based on polycaprolactone (PCL), enriched with bioactive microparticles (MPs) to stimulate angiogenic and osteogenic processes.

METHODOLOGY

Different PCL scaffolds (PCLA, PCLB, PCLC) with bioactive MPs developed in our group were 3D printed (3D Discovery, RegenHU) with high interconnected porosity. The degradation behavior of scaffolds was evaluated in vitro under physiological conditions (HEPES 10mM, pH 7.4, 37ºC) for one year. The viability of human gingival fibroblasts (hGFib) and human mesenchymal stem cells (hMSC) seeded on scaffolds was assessed by Alamar Blue, and imaging. Cytotoxicity (LDH assay) and the expression of different proteins (ELISA) related with angiogenesis and osteogenesis were also evaluated. The in vivo performance of these scaffolds was studied using an in vivo subcutaneous mice model. In order to assess a correct fit of the personalized implants, 3D printed prototypes were tested with polyamide models kindly provided by AVINENT® Implant System.

RESULTS

SEM and MicroCT images showed homogeneous MPs dispersion and macroporosity of 3D printed scaffolds. Minimum scaffolds weight loss was observed after one year. Confocal images indicated complete colonization of scaffolds by hGFib. Similarly, good biocompatibility was observed in hMSC cultures. Analysis of protein expression by ELISA showed an increase in the levels of vascular endothelial growth factor (VEGF) which is related to neovascularization promotion. In the in vivo studies, an absence of acute inflammation and complete tissue integration were observed, indicating scaffold biocompatibility. Furthermore, blood vessels infiltration through scaffolds porosity was identified after one-month implantation. Moreover, personalized prototypes were successfully 3D printed from clinical cases and studied with polyamide bone defects models, obtaining a proper fit of the implant to the defect site.

CONCLUSION

This work shows a promising alternative to the use of metallic meshes, with bioactive and

biodegradable materials to offer a personalized solution for GBR, avoiding their main drawbacks. Additional in vitro assays and an in vivo calvaria study are ongoing to support the results achieved.

REFERENCES

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ACKNOWLEDGMENTS

This project is being developed in collaboration and with funding from AVINENT® Implant System. Authors would like to thank AGAUR for founding with “Doctorat Industrial” grant (2017DI076) and Dr. Elena Xuriguera from the University of Barcelona for her help in performing mechanical testing.

keywords: 3D Printing, Guided Bone Regeneration, Implant, Biodegradable

94238129044

BONE REGENERATION EXPLOITING CORTICOPERIOSTEAL TISSUE TRANSFER FOR SCAFFOLD-GUIDED BONE REGENERATION

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Introduction: Contemporary reconstructive approaches for critical-sized bone defects carry significant disadvantages. As a result, clinically driven research has focused on the development and translation of alternative therapeutic concepts. Scaffold guided tissue regeneration (SGTR) is an emerging technique to heal critical-sized bone defects. However, issues synchronising scaffold vascularisation with bone-specific regenerative processes currently limit bone regeneration for extra-large (XL, 19cm3) critical-sized bone defects. To address this issue, we developed a large animal model that incorporates a corticoperiosteal flap (CPF) for sustained scaffold neo-vascularisation and bone regeneration. Methodology: A pre-clinical evaluation using a 3D-printed medical-grade ε-polycaprolactone b-tricalcium phosphate (mPCL-TCP) scaffold combined with a cortico-periosteal flap (CPF) was undertaken in ten sheep with a medium (M, 9.5 cm3) volume segmental defect of the tibia. Results: In ten sheep the efficacy of this approach for healing M volume segmental bone defects was demonstrated by plain radiography, micro-computed tomography, scanning electron microscopy, immunohistochemical and histological analysis. Furthermore, in two sheep we demonstrate how this approach can be safely extended to heal XL critical- size defects. Conclusion: This study presents an original CPF technique in a clinically relevant and well described pre-clinical model which can be used in conjunction with the SGTR concept to address challenging critical-sized bone defects in vivo.

keywords: bone tissue engineering, critical-sized bone defects, FDA-approved, large animal models, PCL

20941803339

MENISCUS REGENERATION OF THE FUTURE. FROM THE SLAUGHTERHOUSE, THROUGH CELL CULTURE TO 3D BIOPRINTING.

Adam Mieloch (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznan, Poland), Filip Porzucek (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznań, Poland), Monika Mankowska-Wozniak (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznań, Poland), Julia Semba (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznan, Poland), Kamil Kisiel (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznan, Poland), Jakub Rybka (Center for Advanced Technology, Adam Mickiewicz University in Poznan, Poznan, Poland)

The knee meniscus plays an indispensable role in articular surface protection, shock absorption, and stress transmission. Meniscus injuries are extremely prevalent, with an annual incidence of 66 to 70 per 100,000 people. Due to limited vascularization, the regenerative capacity of the meniscus is relatively low and restricted to the most vascularized outer regions. The most commonly performed treatment involves suturing or removal by partial or total meniscectomy. However, meniscectomy significantly increases the incidence of osteoarthritis (OA) later in life by elevating the contact pressure on the tibial plateau. Approximately 50% of patients with meniscal injuries develop OA between 10 and 20 years after the injury. Therefore, optimal treatment options should preserve or mimic the mechanical properties of the meniscus.
3D bioprinting belongs to the family of additive manufacturing (AM) processes that utilize computer-aided design (CAD) for the generation of 3D models through layer-by-layer deposition. The constructs are printed with bioink comprised of viable cells, biomaterials, and additional biological substances. These artificial, cell-laden scaffolds promote and support new tissue formation by providing a suitable environment for cell migration, proliferation, differentiation, and ensuring a proper extracellular matrix (ECM) secretion.
The presentation is focused on a 3D bioprinting-based approach to regenerative medicine of the meniscus. It will also highlight the process of an ECM-based bioink formulation utilizing supercritical CO2 extraction, and a custom-made bioprinting tool on a 6-axis robotic arm.

Acknowledgments: This work was supported by the National Centre for Research and Development TECHMATSTRATEG-III/0027/2019-00 grant.

keywords: applied biotechnology, bionanotechnology, 3D bioprinting, tissue engineering,

S04
3D Writing Within Suspension Media for Tissue Engineering and In Vitro Modeling
Room: S4 B
(28 Jun 2022, 15:30 - 17:00)

Conveners:
Rui M. A. Domingues; Manuela E. Gomes

31451706447

BIOPRINTING HIGH CELL-DENSITY TISSUE MODELS THROUGH SPHEROID FUSION IN SELF-HEALING HYDROGELS

Andrew Daly (Biomedical Engineering, and CÚRAM, at the National University of Ireland, Galway, Ireland, Galway, Ireland)

Cellular models are needed to study human development and disease in vitro, and to screen drugs for toxicity and efficacy. Current approaches are limited in the engineering of functional tissue models with requisite cell densities and heterogeneity to appropriately model cell and tissue behaviors. This talk will describe how spheroid bioprinting in suspension baths can be used to engineer high-cell density microtissues of prescribed spatial organization. Example applications of this technology will include bioprinting induced pluripotent stem cell-derived cardiac microtissue models with spatially controlled cardiomyocyte and fibroblast cell ratios to replicate the structural and functional features of scarred cardiac tissue that arise following myocardial infarction, including reduced contractility and irregular electrical activity. It will also describesd how these models can be used for screening miRNA therapeutics targeting cardiomyocyte proliferation for cardiac regeneration. Recent advances in bioprinting high-cell density tissues with controlled cellular organisation will also be described. These methods are useful for a range of biomedical applications, including the development of precision tissue models with advanced physiological relevance.

keywords: Bioprinting, spheroids, suspension baths, disease models, organoids

20941802324

3D PRINTED ANISOTROPIC AND POROUS DENSE COLLAGEN HYDROGELS TO MODEL SKELETAL MUSCLE EXTRACELLULAR MATRIX

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*Introduction: Despite the crucial role of the muscle extracellular matrix in the organotypic organization and the transmission of mechanical force, most 3D muscle models do not mimic its specific characteristics, namely its biochemical composition, stiffness, anisotropy and porosity. In vivo, muscle extracellular matrix possesses specific characteristics such as a high amount of aligned collagen I to create an anisotropic structure with a significant porosity and a suitable stiffness. Recent approaches of muscle models used non porous hydrogels fabricated from low concentrated collagen to encapsulate muscle cells. Hence, the in vivo properties are not reproduced. Here, we developed a 3D printed collagen hydrogel that mimic the muscle extracellular matrix, i.e collagen anisotropy, adequate stiffness and two ranges of porosity (one to ensure nutrients and oxygen diffusion and the other for cell cultivation).

*Methodology: Dense collagen solutions (30 mg.ml-1) were printed through the 23G flat bottom needle inside a buffer bath. The extrusion process aligned the collagen molecules along the axis of extrusion. The buffer bath played two major roles: it “froze” the collagen alignment and triggered collagen gelling. The printing process was performed unidirectionally for each layer to create an intrinsic porosity between the different collagen filaments. After a rapid period of collagen gelling, needles were introduced within the hydrogel to generate large pores. An additional gelling period was performed to tune the mechanical properties. C2C12, murine skeletal muscle cells were then seeded within the printed hydrogels to evaluate the cell colonization, myotube formation and organotypic organization.

*Results: By tuning the extrusion speed and the gelling process, a 3D printed hydrogel with aligned collagen fibers was obtained. A combination of two gelling strategies (24h PBS 5X + 24h NH3) was optimal to obtain both anisotropy and adequate mechanical properties (E=10 kPa). Scaffold anisotropy was obtained at two different scales: all filaments were printed in the same direction (macroscopic) and collagen fibers were aligned inside printed filaments (microscopic). Concerning the porosity, changing the height between two successive layers allowed to create an intrinsic porosity from 50 to 150 µm. Interestingly, the generation of 100 µm pores preserved the scaffold cohesiveness. This porosity is suitable for nutrients and oxygen diffusion in the whole scaffold, thereby favoring cell viability. Larger pores created by needles molding generated straight channels of 600 µm in diameter. These were easily colonized by C2C12 cells mixed

with Matrigel® to create a suitable 3D environment. After 4 days of differentiation, aligned multinucleated myotubes were formed. Immunostaining with sarcomeric heavy chain myosin revealed the cell commitment into mature myotubes.

*Conclusions: In this study, we developed a 3D printing technique to create a biomimetic muscle extracellular matrix suitable for muscle cell differentiation and cultivation. Our approach focused on the extracellular matrix and its key parameters since it is deeply involved in muscular functions. Hence, this model could be used with patients cells to study and have a better understanding on muscular dystrophies.

keywords: collagen, 3D printing, anisotropy, porosity

94238106505

CHEMICALLY FUNCTIONALIZABLE AND MECHANICALLY TUNABLE BIOMATERIAL FOR EMBEDDED 3D BIOPRINING

Malin Lea Becker (Leijten Laboratory, Department of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands, Enschede, Netherlands), Maik Schot (Leijten Laboratory, Department of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands, Enschede, Netherlands), Roos Uijthof (Leijten Laboratory, Department of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands, Enschede, Netherlands), Jeroen Leijten (Leijten Laboratory, Department of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands, Enschede, Netherlands)

INTRODUCTION: Embedded 3D bioprinting is a promising approach to engineer complex tissues such as patterned or pre-vascularized tissue constructs1,2. However, the resulting tissue constructs are often mechanically weak, unable to form mechanical or chemical gradients, and lack on-demand tunability. Here, we report on dual crosslinkable dextran-based hydrogel as a hydrogel bath for embedded bioprinting, which uniquely allows for local on-demand functionalization as well as formation of spatially controlled chemical and mechanical gradients within printed tissues.

METHODS: Dextran was functionalized with tyramine and biotin moieties to create a dual crosslinkable polymer3. Physically crosslinked embedding baths were created via biotin/avidin protein/ligand interaction. A gelatin or PEG based sacrificial bioink was extruded into the hydrogel using an Inkredible+ 3D printer. Covalent enzymatic or photo-initiated crosslinking of the printing bath was used to create mechanically robust tissues. The tissue's biotin moieties were subsequently used for on-demand biochemical functionalization of the bulk and/or the channel surfaces.

RESULTS & DISCUSSION: Rheological characterization of the physically crosslinked bath revealed shear-thinning and self-healing properties that were highly suitable for embedded bioprinting. Covalent crosslinking resulted in a three-fold increase of the storage modulus of the hydrogels, which enabled the stabilization of printed channel networks, while diffusion of crosslinking agents from the ink resulted in controllable stiffness gradients in the bulk. Without photocrosslinking the bulk, tubular structures were created via enzymatic inside-out crosslinking. Here, tube diameter and channel wall thickness could be independently controlled through variation of the printing speed and crosslinker concentration, respectively. Furthermore, the ink/bath interface allowed for one-step functionalization by loading the ink with biotin-coupled cell bioinstructive moieties.

CONCLUSION: We report on a novel and dual crosslinkable hydrogel suitable for embedded bioprinting, which offers mechanical stability, mechanical tunability, with on-demand biochemical functionalization of tubular and pre-vascularized engineered tissues.

ACKNOWEDGEMENTS: Financial support was received from the European Research Council (ERC, Starting Grant, #759425) and the Dutch Research Council (NWO, Vidi Grant, #17522).

REFERENCES:
1 Lee, A. et al., Science 365, (2019).
2 Highley, C. B. et al., Adv Mater 27, (2015).

3 Kamperman, T. et al., Nat Commun 10, (2019).

keywords: embedded, 3D printing, vascularization, bioinstructive

31412732346

AN OPEN SOURCE EXTRUSION BIOPRINTER BASED ON THE E3D MOTION SYSTEM AND TOOL CHANGER TO ENABLE FRESH AND MULTIMATERIAL BIOPRINTING

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Bioprinting is increasingly used to construct complex 3D cell models and tissue constructs for in vitro studies, and its capacity to produce transplantable tissues is being intensely explored. However, progress in these fields could be further accelerated by increasing the access to easy-to-use open source bioprinters.
Here we describe an open source extrusion bioprinter based on the E3D motion system and tool changer which enables high resolution multimaterial bioprinting.
The E3D motion system and tool changer was adapted to control the position of a custom 3D-printed syringe pump extrusion tool, equipped with a stepper motor to actuate the plunger and regulate bioink extrusion. The bioprinter is housed in a polycarbonate enclosure equipped with a HEPA filter to reduce the risk of contamination during printing. The versatility of the bioprinter was demonstrated by creating collagen constructs using the freeform reversible embedding of suspended hydrogels (FRESH) method (1), as well as printing multimaterial constructs composed of distinct sections of laminin and collagen bioink. Image analysis of cell viability dyes was used to evaluate the capacity of the bioprinted constructs to support survival of breast cancer cells following direct seeding onto printed constructs or after printing in cell-laden bioinks, and was assessed after short-term (24 h) and long-term (1 wk) incubations (2).
The syringe pump extrusion tool is compatible with different syringe volumes, and needles and nozzles of different calibres, and we determined that narrow linear features could be accurately reproduced (100 ± 12 mm) when extruding bioinks through a 50 mm needle. The dimensions of FRESH and multi-material printed constructs proved faithful to their intended designs, and a high degree of cell viability was seen for cells seeded onto collagen or dispersed in laminin bioinks following both short- and long-term incubations. Furthermore, cell death could effectively be studied in cells grown on FRESH printed collagen constructs following exposure a known apoptosis-inducing agent.
This open source bioprinter is easily adapted to the specific needs of various bioprinting applications; for example, creating small-scale 3D tumor tissue constructs for the purpose of drug-screening. The bioprinter solution presented here is versatile, easy-to-use, and the motion system is already supported by open source data, which together offer an accessible entry point to the novel and rapidly expanding field of bioprinting.

References:

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2 Engberg, A., Stelzl, C., Eriksson, O. et al. Sci Rep 11, 21547 (2021).

keywords: open source, bioprinting, FRESH bioprinting, E3D motionsystem, breast cancer model

83767218366

HIGH RESOLUTION LIGHT-BASED 3D PRINTING OF CELL-LADEN BIO CONSTRUCTS

Jorge Madrid-Wolff (EPFL, Lausanne, Switzerland), Antoine Boniface (EPFL, Lausanne, Switzerland), Damien Loterie (Readily 3D, Lausanne, Switzerland), Paul Delrot (EPFL, Lausanne, Switzerland), Christophe Moser (EPFL, Lausanne, Switzerland)

3D printing has revolutionized the manufacturing of volumetric components and structures in many areas. Several fully volumetric light-based techniques have been recently developed thanks to the advent of photocurable resins, promising to reach unprecedented short print time (down to a few tens of seconds) while keeping a good resolution (around 100 microns). However, these new approaches only work with homogeneous and relatively transparent resins so that the light patterns used for photo-polymerization are not scrambled along with their propagation. Herein, we propose a method that takes into account light scattering in the resin prior to computing projection patterns. Using a tomographic volumetric printer, we experimentally demonstrate that implementation of this correction is critical when printing objects whose size exceeds the penetration depth of light. To show the performances of the scattering corrections we fabricate cell-laden hollow constructs that would be difficult to print otherwise because of light scattering by the cells. Bioprinting cm-scale hollow constructs is therefore challenging but also crucial because hollow channels allow for the inflow of nutrients and oxygen to the cells deep inside the hydrogel. Based on a fine characterization of the scattering process, the proposed scattering correction spatially redistributes light to avoid over-polymerization and clogging the channels, more light is sent to the fine features of the edges while less light is sent to the bulk of the construct. As an example, this technique allowed us to fabricate in 36 seconds a cm-scale construct with four millimetric channels unclogged and a solid core in a soft hydrogel loaded with 4 million HEK 293 cells mL-1. As a comparison, the same printer without the scattering correction yielded clogged channels and a void core. Previous reports have demonstrated the fabrication of similar structures under concentrations of only 10 000 or 1 million cells mL-1 using similar printing technologies. To conclude, this scattering correction extends the capabilities of conventional light-based volumetric printing and opens up promising perspectives in printing inside turbid materials with particular interesting applications for bioprinting cell-laden constructs.

keywords: 3D bio-printing, Photopolymerization, Light scattering, Cell-laden constructs

83767224366

MAGNETICALLY-ASSISTED 3D BIOPRINTING OF TISSUE ENGINEERED TENDONS

Alberto Pardo Montero (3B's Research Group, Barco, Guimaraes, Portugal), Syeda M. Bakht (3B's Research Group, Barco, Guimarães, Portugal), Manuel Gómez-Florit (3B's Research Group, Barco, Guimarães, Portugal), Simão P. B. Teixeira (3B's Research Group, Barco, Guimarães, Portugal), Rui M. A. Domingues (3B's Research Group, Barco, Guimarães, Portugal), Manuela E. Gomes (3B's Research Group, Barco, Guimarães, Portugal)

Introduction:
Tendon tissues have highly-anisotropic physical properties that are responsible for its biomechanical performance and biological organization. The recreation of its 3D extracellular matrix (ECM) and cellular patterns in bioengineered constructs remains challenging. The concept of magnetically-assisted 3D bioprinting with magnetic hydrogel bioinks can be exploited to fabricate anisotropic scaffolding materials with 3D architectures that resemble the organization of tendinous ECM and to modulate biophysical/biochemical cues that influence the fate of encapsulated cells. Moreover, magnetic nanoparticles (MNPs) remote response enables their use as magnetomechanical actuators to control cellular/tissue behavior. However, a main challenge hindering the implementation of this concept is how to control the 3D organization of magnetic elements during layer-by-layer printing without compromising the fidelity and resolution of printed constructs. To overcome this dichotomy, here we combine the concepts of magnetically and matrix-assisted 3D bioprinting technologies. This strategy enables to fabricate high-resolution constructs with magnetic bioinks that remain liquid for long enough before gelation to allow the orientation of magnetic elements, thus building 3D fibrillar patterns resembling the microstructure of tendon tissues.

Methodology:
Monodisperse iron oxide-based MNPs displaying extremely-high magnetization values were synthesized through thermal decomposition. These MNPs were then incorporated into electrospun polycaprolactone meshes, which were subsequently cryo-sectioned at different lengths to produce dispersed magnetic microfibers. Magnetically-responsive bioinks were prepared by mixing the magnetic short fibers with gelatin solutions and human adipose-derived stem cells (hASCs). The 3D extrusion bioprinting steps were performed under the presence of fairly uniform external magnetostatic fields produced by a two parallel magnets setup. Agarose and cellulose nanocrystals (CNCs)-based fluid gels (supplemented with transglutaminase for gelatin crosslinking) were tested as support baths.

Results:
Zinc-doping demonstrated to be the most efficient approach to increase the magnetic power of superparamagnetic iron oxide-based MNPs. Zn-Fe_3O_4 MNPs were used to prepare magnetically-responsive electrospun polycaprolactone microfibers with 20-100 µm of length. The incorporation of these microfibers and hASCs in gelatin solutions resulted in bioinks that enabled the fabrication of high-resolution 3D-printed constructs when using CNCs as suspension baths, but not when with the respective granular agarose gels. Exploiting the high magnetic power of the MNPs, very low particle concentrations and weak magnetic field strengths were enough to align the fibers during the layer-by-layer extrusion printing steps. The anisotropic microstructure of the biomimetic constructs induced elongated growth and phenotypic commitment of the encapsulated cells.

Conclusions:
Our strategy allows the 3D manufacturing of biomimetic magnetic constructs that replicate the architecture of native tendons ECM. We established the design of MNPs with extremely-high magnetic power as a key factor to fabricate bioink hydrogels that can be manipulated using low contents of magnetic material and weak magnetic fields, minimizing the toxic/safety risks associated with these factors. The combination of magnetically-assisted 3D bioprinting strategies with the use of CNCs support baths has demonstrated to be essential for enabling the proposed concept. The resulting anisotropic 3D fibrillar microstructure of the printed constructs revealed effective on directing encapsulated cell fate. The effects of remote magnetomechanical actuation on cellular constructs is currently under investigation.

Comment: Acknowledgements: EU HORIZON 2020 for projects ACHILLES (H2020-WIDESPREAD-05-2017-Twinning-810850) and MagTendon (ERC-2017-CoG-772817); FCT/MCTES for scholarships PD/BD/129403/2017 (M.B.) and PD/BD/143039/2018 (S.P.B.T.) under PhD PATH (PD/00169/2013), for project SmarTendon (PTDC/NAN-MAT/30595/2017), and individual contracts 2020.03410.CEECIND (R.M.A.D.) and CEECIND/01375/2017 (M.G.F.); Xunta de Galicia for postdoctoral grant ED481B2019/025 (A.P.).
Authors declare no conflicts of interest.

keywords: tendon tissue engineering, anisotropic biomaterials, magnetic stimulation, magnetically-assisted 3D bioprinting

31412756349

DEVELOPMENT OF BIOPRINTED OSTEOCHONDRAL TISSUE: AN IN-VITRO MODEL FOR DRUG DISCOVERY

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Introduction: Osteochondral (OC) disorders like osteoarthritis (OA) and rheumatoid arthritis (RA) damage the joint's cartilage and subchondral bone. Their treatment remains a significant challenge for both researchers and orthopedics. In vitro models of OC tissue have become an essential tool to help investigate pathogenesis, develop drug screening, and test potential therapeutic approaches. This study aims to create a bio-printed OC construct recapitulating the bone and cartilage compartment as drugs testing platforms.
Methodology: Two different hydrogels, including a blend composed of gelatin methacrylate (GelMA) with nanosized hydroxyapatite (nHA) and tyramine-modified hyaluronic acid (THA), were selected for the bioprinting of bone and cartilage tissue mimics. The composition of GelMA hydrogel (10% w/v) with different concentrations of nHA (1-10% w/v) and THA with concentrations of 2.5-5% w/v were characterized by rheology and their cytotoxicity was assessed via live-dead assay. Later, the pre-differentiated osteoblast and endothelial cells were encapsulated into GelMA-nHA and micropellet chondrocytes into THA hydrogels for bioprinting osteochondral construct. After 2 weeks of culturing, the successful generation of OC tissue was confirmed by real-time RT-PCR and histology.
Results: The storage modulus (G') of all GelMA/nHA hydrogels was significantly higher than GelMA, however, there was no significant difference in G' values for the GelMA/nHA as a function of added nHA. Due to the know temperature sensitivity of GelMA, a rheological temperature sweep and series of printing tests were performed to establish a suitable printing temperature, which was confirmed to be 20°C, independent of the addition of nHA. Calcein-AM (Ca-AM) and Ethidium Homodimer-1 (EthD-1) staining for GelMA (10% w/v) with three concentrations of nHA (1, 3, and 5% w/v) at 2, 24, 72, and 168 h after printing showed the percentage of living cells after 72h in GelMA containing 3 and 5% (w/v) nHA was less than 50%, while in GelMA with 1% (w/v) nHA it remained high (>95%) even after 168h. Therefore, this formulation was chosen for the subsequent generation of bone tissue mimic.
Shear flow curves of THA hydrogels showed an increase in viscosity as a function of THA concentration. The damping factor, which is a ratio between the loss modulus G'' and the storage modulus G', has been shown to be directly related to the extrudability[1]. The calculated damping factors for each concentration of THA (%w/v) (THA 2.5%= 0.4947 ± 0.038, THA3.5%= 0.5935 ± 0.012, and THA5%= 0.7391 ± 0.039), indicated that THA3.5%(w/v) was in the printable range. Cell viability assays for THA hydrogels showed a high percentage of living cells for THA 3.5% (w/v) compared to THA 5% (w/v) after 168 h. Based on cell viability assay, viscosity, and printability, a 3.5%(w/v) concentration of THA was selected for generating cartilage tissue mimic part.

Conclusion: We developed GelMA-nHA and THA hydrogels for bone and cartilage parts respectively. We also optimized printing parameters based on printability and shape fidelity and cell density according to cell viability for bioprinting OC constructs.

[1] Petta et.al, 2018, ACS Biomater. Sci. Eng, DOI: 10.1021/acsbiomaterials.8b00416.

keywords: Osteochondral tissue, Bioprinting, Hydrogels, Rheology, Drug discovery

S05
Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice
Room: S3 A
(30 Jun 2022, 11:00 - 12:30)

Conveners:
Veronika Hruschka; Mohammad Alkhraisat

83871206328

BETWEEN RISK, PRIVACY AND MAGIC: REGULATORY AND REIMBURSEMENT OF INDIVIDUAL REGENERATIVE IMPLANTS

Daniel Seitz (BioMed Center Innovation gGmbH, Bayreuth, Germany)

Regenerative implants are most ideally suited for individualized production using additive manufacturing. For regulatory market access, if not containing cells or tissue compounds making them ATMP's, the choice is between serially produced scaffolds, patient-adapted designs or custom-made devices that require a prescription. While it seems helpful that custom-made products do not get a CE mark and leave much of the risk with the surgeon, the procedure is cumbersome to the clinical staff and reimbursement often prone to discussion. This might be the reason why entire sets of prefabricated size-ranges are still taken into the sterile area every day. In other regulatory spheres like Australia and USA, more practical approaches towards medical product registration of AM implants exist, while the MDR relies on the freshly enhanced custom-made path. A more viable solution for the European regenerative implant market that fits into reimbursement seamlessly might unleash the potential of AM in modern medicine, a field in which Europe is struggling to maintain its leading position.
With the current situation, and despite all the hype, AM regenerative implant manufacturers have to feel rather exotic and miss a secure embedding in the daily clinical routine, despite all the magic they can produce especially in cooperation with the right clinical partner, usually a university hospital. The discrepancy stems from traditional CE marking as medical product registration path, simultaneously creating clinical evidence or at least acceptance and thus the base for reimbursement by public and private health plans. Individually manufactured implants do not fit into this registration scheme, making it necessary to defend their application from case to case. However, it appears that, by copying from software medical product registration, and proving efficacy and safety similar to patient-adapted implants, a more pragmatic regulatory approach would be possible. Today, the development of AM production chains has come to a point where an individual implant, designed by constraints from the treating medical authority, can be produced and delivered on the push of a button. It seems possible that reimbursement, be it for operational models, implants or tissue engineering constructs, can be equally integrated into daily clinical practice – making the magic regulated and risk controlled.
An important aspect remains data handling and privacy in the light of the GDPR. First mobile solutions for something as simple as CT data transfer are emerging. A standardization of data handling, respecting the patient's private sphere and at the same time gathering information important for AM on gender, personal heritage and behavioral patterns in a modern way might even be the base for a sound regulatory scheme, if integrated into intelligent information management as integral part of AM regenerative implant design.

keywords: additive manufacuture, medical product registration, reimbursement, data handling

94355103928

MEDICAL ADDITIVE MANUFACTURING: IS IT READY FOR BROAD CLINICAL USE?

Francesco Moscato (Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria)

Medical additive manufacturing (aka medical 3d-printing) has received increasing attention in the past years with research applications in many clinical domains, particularly in surgical disciplines (e.g. orthopedic, oral, cardiovascular). In some domains medical 3d-printing has established itself in daily clinical practice, however the question remains if is it ready for broad clinical use. In this talk a short survey about the state-of-the-field will be presented, followed by examples from several research & development projects at the Medical University of Vienna. The selected projects will highiligh the medical background, the technological options employed as well as the challenges we have been encountering towards a successful establishment of 3d-printing in the clinics.

Comment: Please include these affiliations:

1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria
2) Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria
3) Austrian Cluster for Tissue Regeneration, Vienna, Austria

keywords: medical additive manufacturing, translational medicine, surgical planning and training, custom made implants

52354514949

3D BIOPRINTING OF STRUCTURALLY ORGANIZED MENISCUS TISSUE

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Introduction
Additive manufacturing approaches have the potential to address a number of major challenges in the field of meniscus tissue engineering (TE), in particular the development of anatomically defined grafts with a spatial architecture and composition mimetic of the native tissue. Here, we report a novel method to engineer organized soft tissues, with a collagen architecture and mechanical behaviour similar to native meniscus. We compared the capacity of two direct material writing techniques, specifically fused deposition modelling (FDM) and melt electrowriting (MEW), to generate guiding structures for cells that are deposited using inkjet bioprinting. We hypothesised that by fabricating polymeric scaffolds with specific architectures it is possible to control collagen fibre organization and the mechanical behaviour of the engineered fibrocartilaginous tissue, thereby better recapitulating the native meniscal tissue

Materials and methods
FDM and MEW were employed to fabricate polycaprolactone (PCL) scaffolds with various defined geometric architectures. Furthermore, large volume MEW scaffolds with micro-fibrous features were fabricated to replicate the complex wedge-shaped macro-architecture of the human meniscus. A custom alginate based bioink was developed and inkjet bioprinting was used for the dispensing of cell-laden bioinks into scaffolds. After inkjetting, the cells were cultured in presence of TGF-β3 to induce chondrogenesis. Mechanical testing was conducted to determine the compressive and tensile properties of the engineered tissues.

Results
First, we assessed the suitability of the developed bioink for cartilaginous TE applications, finding that its rapid degradation allows cells to condense and begin the process of generating new tissue while exhibiting high levels of cell viability. The multicellular aggregates which formed within the defined boundaries provided by the PCL fibres generate a neo-tissue where the organization is determined by the architecture of the scaffold. Both FDM and MEW fibrous architectures facilitated the formation of anisotropic collagen networks resembling those of the native meniscus. However, PCL scaffolds fabricated using FDM displayed mechanical properties that are unsuitable for meniscus replacement, as they are too stiff in compression. By using MEW as a fabrication technique, the mechanical strength of the engineered tissues after 5 weeks of in vitro culture was similar to the native tissue. After demonstrating the benefit of using MEW to create scaffolds for meniscus regeneration, we fabricated larger (5 mm height) scaffolds replicating the shape of the meniscus. The pore architecture of these large scaffolds remained open even at the highest sections, enabling the bioprinting of cells and facilitating tissue growth throughout the entire scaled-up construct.

Discussion
In the present work, we have successfully developed a biofabrication approach that allows precise control over the orientation of the deposited collagen tissue. By using MEW as a fabrication technology we could better mimic not only the collagen network architecture but also engineer tissues with similar anisotropic mechanical behaviour. We have also succeeded in the fabrication of large volume MEW scaffolds of up to 5 mm height with well-defined micro-

fibrous and macro-architectural features. This work demonstrates the potential of integrating MEW and bioprinting to engineer structurally organised soft tissues.

keywords: Biofabrication, MEW, Meniscus

20941813806

COMPUTATIONAL MODELLING OF MECHANICAL PROPERTIES OF THE SCAFFOLDS PRODUCED BY MELT ELECTROWRITING

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Introduction:
Melt Electrowriting (MEW) is a novel 3D printing method that allows fabricating scaffolds with different designs, including structural gradients. The gradient scaffolds can be useful, for example, in the engineering of tissue interfaces, which are characterized by gradually changing mechanical and biological properties [1]. Nonetheless, the prediction of such scaffolds' properties is a challenge.
The aim of this project is the development of a computer simulation that will allow predicting the mechanical properties of the scaffolds produced with MEW. This will ultimately facilitate the designing process for researchers working with MEW.

Methodology:
Polymeric scaffolds were fabricated using the MEW technique using polycaprolactone. the mechanical properties of scaffolds with different pore sizes and designs were analyzed in tensile testing. COMSOL Multiphysics® was used to simulate the tensile testing of printed scaffolds.

Results:
The scaffolds with varying designs, including gradients, were printed with a fiber diameter of 16 μm. SEM images showed good accuracy of printing and high precision of layer deposition. Tensile test results revealed the dependency of mechanical properties on scaffold design. The developed computational models allowed for accurate simulations of scaffolds' mechanical performance, i.e. Young's Modulus and deformation.

Conclusions:
The mechanical properties of MEW printed scaffolds can be tuned by the scaffold's design. The proposed computer simulation helps predict the mechanical properties of the scaffolds with high accuracy, at the deformation range of up to 2% (elastic region). In the next steps, computer simulations will be evaluated for higher deformations. Such models will accelerate the development of MEW scaffolds with tissue-specific properties.

[1] Bayrak E, Huri PY. Engineering Musculoskeletal Tissue Interfaces. Frontiers in Materials 5, 24, 2018

keywords: Melt Electrowriting, gradient scaffolds, computer simulations, PCL, tissue engineering

62825410404

DEVELOPMENT OF AN ELECTROCONDUCTIVE, 3D-PRINTED SCAFFOLD DESIGNED TO PROMOTE AXONAL REGROWTH AFTER SPINAL CORD INJURY

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Introduction
Spinal cord injury (SCI) induces paralysis by severing the long axons of neurons and recovery is inhibited by poor regrowth rates. As neural cells are electroactive, electrical stimulation (ES) may present a promising method of promoting axonal regrowth when applied in conjunction with electroconductive (EC) biomaterials1. To efficiently deliver ES to regrowing motor and sensory axons, it is essential to have precise control of scaffold geometry.
This work focused on producing novel EC scaffolds for spinal cord injury by coating 3D-printed polycaprolactone (PCL) with polypyrrole (PPy) and assessing its suitability as a substrate for neuronal growth.

Methodology
PCL scaffolds consisting of multiple interlocking 'axon' channels were 3D-printed (Allevi 2 printer). PPy was then polymerised in situ to form an EC coating2. Electroconductivity was measured via the 4-point method and surface morphology and coating thickness were assessed using SEM.. Biocompatibility was tested by seeding SH-SY5Y neurons on PPy/PCL films and measuring metabolic activity and total cellular DNA. Neurons were immunostained for beta-III tubulin, counterstained with DAPI for nuclei and imaged using a Nikon 90i fluorescent microscope to determine neurite outgrowth. Images were analysed using ImageJ.

Results
A method was developed to coat complex 3D-printed PCL structures with a biocompatible, EC PPy layer. SEM images of coated films show that PPy forms a network of particles over the PCL surface. Conductivity of the PPy coating was 15 ± 5 S/m, rendering the scaffold suitably electroconductive for biological applications. In cultures of SH-SY5Y neurons on 2D PCL and PPy/PCL films, metabolic rate and total cellular DNA increased significantly ($p<0.05$) in both groups between day 1 and 3, with no significant difference in either metric between groups, showing the PPy coating is as biocompatible as uncoated PCL, providing a suitable substrate for neural proliferation. Neurons cultured on both film types (7 days) exhibited robust neurite outgrowth and typical morphology with no significant difference in cell number or neurite length between groups, confirming neuronal viability.

Conclusion
Biocompatible, 3D EC scaffolds with complex architectures were produced for SCI repair. Coating 3D-printable PCL with EC PPy allows printing of biocompatible EC structures with precise, controlled geometries and porosities that can match the organisation of grey and white

matter tracts in the cord. Conductivity of the PPy coating is over 30 times higher than central nervous system grey matter, and 8 times higher than cerebrospinal fluid, potentially allowing for efficient direction of electrical stimulation3. Taken together, these data indicate that PPy/PCL is biocompatible, supports neuronal growth and is a suitable substrate for growth of spinal cord neurons. Ongoing optimisation work will determine the ability of the EC scaffold to increase efficacy of ES to further promote neurite extension as a prerequisite for SCI applications.

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ACKNOWLEDGEMENTS
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keywords: 3D-Printing, Electroconductive Materials, Polymers, Nerve Repair

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MULTI-MATERIAL 3D PRINTING OF CERAMICS FOR FABRICATING BI-PHASIC IMPLANTS

Martin Schwentenwein (Lithoz GmbH, Vienna, Austria), Sebastian Geier (Lithoz GmbH, Vienna, Austria), Serkan Nohut (Piri Reis University, Istanbul, Turkey)

Combining different materials or material-properties in 3D printing is garnering widespread attention due to the wide range of possibilities that it provides to produce parts which are more functional and have improved properties. This paper presents the combination of lithography-based ceramic manufacturing, a vat photopolymerization technology capable of realizing high resolution 3D printing for ceramics with the mentioned multi-material approach. The presented approach not only enables the combination of different ceramics in different layers of the printed component, but also the spatially resolved combination within the same layer and hence, paves the way to the realization of complex bi-phasic ceramic components. First successful trials that will be presented include the combination of alumina and zirconia-toughened alumina (ZTA) and zirconia and hydroxyapatite. Especially, the latter combination possesses big potential in implants, as it allows combining the mechanical properties of zirconia ceramics with the biological performance and activity of hydroxyapatite. In this way it is possible to create implants that combine different mechanical, chemical, and biological properties in different areas and hence, would be very interesting candidates as bone replacement materials. Moreover, this multi-material 3D-printing approach also allows the localized introduction of different levels of porosity and in such a way functionally-graded properties. Using so-called porogens or fugitives it was possible to create density gradients between almost fully dense areas and areas with a volumetric porosity of around 50%. In combination with the design freedom of 3D printing and the possibility of manufacturing complex cellular or lattice designs, this allows to fabricate implants or scaffolds with hierarchical porosity.
The paper will not only present the actual multi-material 3D printing process, but also focus on the results and current challenges in terms of co-sintering of different ceramic materials. The initial results show that this technological approach holds great potential to path the way from classical single material structures to bi-material components and subsequently multi-material and functionally-graded ceramics.

keywords: additive manufacturing, ceramics, vat photopolymerization, multi-material

52354565457

DESIGN AND EVALUATION OF LATTICE-STRUCTURED MENISCAL IMPLANTS

Disha Tupe (Johannes Kepler University Austria, Linz, Austria), Zoltan Major (Johannes Kepler University Austria, Linz, Austria), Veronika Miron (Johannes Kepler University Austria, Linz, Austria)

Additive manufacturing allows for a wide range of freeform and complex shapes to be made with little or no manufacturing limitations. The most significant benefit of additive manufacturing in medical applications is that it allows for the creation of patient-specific medical products such as implants. Individualized implants are considered to provide greater comfort, precise fit, user acceptance, and may result in fewer revision surgeries. Additive manufacturing allows for tool-less production, which can lower prototyping & tooling costs as well as reduce medical product development time. Many challenges arise while designing for patient-specific implants, as each product has its own distinct characteristics. There is no one-size-fits-all approach that allows to infinitely reproduce the same result as with traditional procedures. A fast design process, verification, and validation of the implant design for the mechanical stability, biocompatibility, and printability, are among the challenges.
The meniscus, a fibrocartilage structure in the knee joint, plays a very significant role in load transmission, shock absorption, lubrication, and nutrient supply to the articular cartilage. Meniscus damage or wear occurs as a result of accidental injuries or aging, and may necessarily require partial or total replacement of the meniscus. This study focuses on the design of individualised meniscal implants that would relieve pain and restore knee joint functionality. The research aims to explore the load bearing capacity of meniscus implants using three different lattice structure designs. Material properties, cell topology and shape, and relative density of structures all influence the properties of lattice structures. In current study, a shell- core type meniscus geometry is analyzed where the lattice structures in core serves as a strengthening component, while the shell binds the meniscus geometry together and keeps it in shape. The beam diameters and lattice size of each individualized implant can be altered to better meet the strength requirements and production constraints. The implant design proposed here could also be used to create a multi-material meniscus implant that combines the strength of different materials.

Comment: Acknowledgement : This research is being carried out as part of a European project called INKplant - Ink-Based Hybrid Multi-Material Fabrication of Next-Generation Implants (Project Number: 953134). We warmly thank Profactor GmbH for coordinating the project and the whole consortium team who provided utmost support and expertise to evaluate the necessary data related to WP6 and WP7 user cases.

keywords: individualized implants, lattice structures, mechanical stiffness

73387302597

PLACING A MEDICAL DEVISE IN THE MARKET: A FOCUS PERSPECTIVE ON THE BIOLOGICAL CHARACTERIZATION OF A MEDICAL DEVICE

Mohammad Alkhraisat (BTI Biotechnology Institute, Vitoria, Spain), Eduardo Anitua (BTI Biotechnology Institute, Vitoria, Spain)

Current medical devices certification is challenging due to the update in the regulatory norms and the appearance of gaps (grey zone) due to advances in both the materials and the fabrication technology. A key aspect when reaching a higher level of technology readiness is to have a comprehensive view of the entire process that will lead to the conformity of the devise with the medical device regulation. To have a focus perspective, it is interesting to work on the research project to have defined several key steps for future medical device certification like the definition of the intended use, the documentation of risk analysis and the iterations of risk management, the documentation of design and manufacturing iterations, and the identification of the materials that are included in the medical device. This will lead to the definition the final device that shall be assessed for the biological characterization for conformity assessment under the medical devise regulation. The ISO 10933 series and the MDR devices that are composed of substances or of combinations of substances are discussed in an integrative approach to provide a focus perspective on the biological characterization of a medical device. Furthermore, the concpect of “equivalent” medical devise could be an interesting option for the additive manufacturing of medical devices. For example, custom-made approach could be used as a transition toward certification of series production of medical devices by additive manufacturing.

keywords: additive manufacturing, MDR, Biological characterization,

S06
Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective
Room: S3 A
(28 Jun 2022, 13:30 - 15:00)

Conveners:
Riccardo Levato; Carlos Mota

31451708649

DYNAMIC HYDROGELS FOR BIOFABRICATION

Matthew Baker (Maastricht University, Maastricht, Netherlands)

The creation of bioinks that can both support cells during the fabrication process and lead to advanced tissue function post-processing remains a challenge. If the tissue engineering community wishes to unlock the potential of 3D biofabrication techniques, new materials must be designed to meet these needs. In our lab, we take a look at new strategies with dynamic hydrogels as a potential solution to the demands of both fabrication and tissue formation. Recently, we have shown the ability to engineer shear-thinning and self-healing hydrogels utilizing dynamic (dynamic covalent and supramolecular) chemistry with rationally controllable mechanical properties. These viscoelastic materials are able to be 3D bioprinted, and offer unique opportunities to control cell behavior or improve culture conditions. Combined, these strategies show a potential path forward for the design of next-generation bioinks and tissue engineering hydrogels.

keywords: hydrogels, bioprinting, biomaterials

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BIOPRINTING ON-CHIP MICROPHYSIOLOGICAL MODELS OF HUMANIZED KIDNEY TUBULOINTERSTITIUM (BIRDIE)

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Chronic kidney diseases (CKD) affect approximately 10% of worldwide population [1]. Currently in vitro models fail to mimic the complexity of the kidney essential for relevant in vitro studies. CKD can be caused by a multitude of factors spanning from nephrotoxic events, especially when exposed to cytotoxic compounds (e.g., antibacterials, corticosteroids, anti-cancer drugs) up to viral infection. Hence, the BIRDIE project aims to develop a novel platform for studying CKD that can support nephrotoxicity and viral testing and in which both structure and function of the native kidney can be mimic. Therefore, BIRDIE combines three different technologies: bioprinting, human pluripotent stem cells (hiPSCs), and organ-on-chip.
Through bioprinting, we will build a three-dimensional organ-like structure where cells can be patterned and spatially organized as in vivo. Secondly, hiPSCs differentiation into kidney progenitors will offer a more reliable cell source compared to the canonical 2D cultures of primary cells or cell lines. Lastly, the introduction of a microfluidic system will recreate the physiological conditions of the native kidney, further sustaining progenitors development. Overall, the in vitro model envisioned in BIRDIE will provide a physiologically relevant platform as a reliable tool to study nephrotoxicity and viral infection associated to CKD, supplanting current in vitro and in vivo models.

Reference
[1] Wilson S, et al., J Clin Hypertens (2021) 23:831–4.

keywords: Kidney, bioprinting, organ-on-chip, in vitro model, nephrotoxicity

83767225448

OPTICALLY-TUNED BIORESINS FOR THE ULTRA-FAST VOLUMETRIC BIOPRINTING OF HEPATIC ORGANOID-LADEN BIOFACTORIES

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Introduction: Developing more predictive in vitro platforms for biomedical research remains a major challenge in tissue engineering. 3D bioprinting allows patterning of cell-laden biomaterials into hierarchical structures. Volumetric bioprinting (VBP) is a novel light-based approach that tackles challenges posed by conventional approaches, through the layer-less biofabrication of viable and highly complex cell-laden structures at unprecedented speeds[1]. Given the requirement of high cell densities to create functional tissue mimics, strategies to overcome the light-scattering effect of intracellular organelles are needed to resolve high-resolution prints. Herein, an optically-engineered bioresin was developed to pattern morphologically-undisrupted organoids into complex centimeter-scale assemblies. Patient-derived human hepatic organoid-laden constructs were printed to create advanced in vitro models that capture salient features of the liver involved in systemic homeostasis and detoxification.
Methods: 405nm light back-filtered projections of a 3D object are directed onto a volume of cell-laden bio-resin (gelatin methacryloyl with visible-light photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate), to selectively crosslink the hydrogel in a spatially-controlled fashion. The resolution of VBP in the presence of hepatic cell line (HepG2) or patient-derived hepatic organoids was enhanced through the addition of refractive index-matching compound iodixanol. These optically-tuned resins were used to print high hepatic organoid densities (up to 107cells/mL). Viability and metabolic activity of bioprinted organoids was evaluated, as well as hepatic differentiation capacity of VBP-prints (hepatic markers, albumin secretion, and cytochrome activity) compared to extrusion bioprinted (EB) constructs. Finally, cell-laden, mathematically-derived architectures with different structural properties were printed at high resolution and cultured under dynamic perfusion to evaluate organoid metabolism of ammonia.
Results: VBP-printed constructs were fabricated in tens of seconds, achieving previously unattained resolutions (41.5±2.9µm positive and 104.0±5.5µm negative features). The concentration of iodixanol was optimized to match the refractive index of intracellular components of both HepG2s and organoids, resulting in a significant resolution enhancement of cell-laden constructs (50.5±6.0µm). Hepatic organoids ranging from 100µm to 1mm in diameter were successfully printed via VBP with high accuracy. Compared to EB-printed structures,

where shear forces resulted in organoid fragmentation and lower viability (73.2±1.2%), VBP-printed organoids exhibited high viability (93.3±1.4%), maintained their morphology and displayed apicobasal polarity post-printing. Complex gyroid-like structures with different pore architectures printed within 16-20s were integrated in a fluidic system and exhibited differences in permeability and surface-area-to-volume ratio. This resulted in enhanced rates of ammonia metabolism (33.5±5.8-24.3±1.4nmol mgtotal protein-1) compared to static controls (12.7±0.3nmol mgtotal protein-1), as well as shape-dependent changes in metabolism.
Conclusion: This study demonstrated the contactless bioprinting of complex and labile biological structures (hepatic organoids) via VBP. Through a refractive index-matching approach, an optically-tuned gelMA resin enabled high-resolution printing of cell-laden structures. Organoids exhibited high viability and hepatic differentiation capacity post-printing. Furthermore, the dynamic culture of convoluted VBP-printed structures was demonstrated through architectures that could modulate organoid function in a shape-dependent fashion. The combination of organoid technology with the ultra-fast printing times and freedom of design offered by VBP shows promise for the development of new predictive platforms for in vitro disease modeling and drug screening.
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Comment: This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 949806, VOLUME-BIO) and from the European's Union's Horizon 2020 research and innovation programme under grant agreement No 964497 (ENLIGHT). R.L and J.M acknowledge the funding from the ReumaNederland (LLP-12, LLP22, and 19-1-207 MINIJOINT) and the Gravitation Program "Materials Driven Regeneration", funded by the Netherlands Organization for Scientific Research (024.003.013). R.L. also acknowledges funding from the NWA-Ideeëngenerator programme of the Netherlands Organization for Scientific Research (NWA.1228.192.105). M.B. and B.S. acknowledge funding from the research program Applied and Engineering Sciences with project number 15498, which is financed by the Netherlands Organization for Scientific Research. J.M.W and C.M acknowledge funding from the Swiss National Science Foundation: "Light based Volumetric printing in scattering resins" (n°200021_196971).

keywords: Volumetric bioprinting, organoids, advanced in vitro models, hydrogels, biofabrication

31412730159

DEVELOPMENT OF CONDUCTIVE STIMULI-RESPONSIVE FIBROUS HYDROGELS FOR NEURAL INTERFACES

Seyed Shahrooz Zargarian (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Chiara Rinoldi (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Yasamin Ziai (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Paweł Nakielski (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Filippo Pierini (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland)

Introduction:
Recently, conductive hydrogels have garnered significant attention and permitted momentous improvements in neuroscience due to their tissue-like softness, chemical steadiness, and sufficient electrical conductivity.(1) They have been utilized as interfaces for neural electrode arrays to improve their biocompatibility and lower protein adsorption. In particular, these materials have the potential to circumnavigate the mechanical mismatch between the neural probes and the implanted tissue.(2) Therefore, the transition from rigid to soft interfaces can improve the performance of the recording/stimulating devices by minimizing tissue irritation and neuronal cell loss. Alas, the chronic application of such interfaces is still challenging due to the poor adhesion of soft hydrogels to metallic electrodes and their relatively low stimuli-responsive characteristics.
The utilization of porous, high surface area and stimuli-responsive hydrogels may compensate for these physiochemical shortcomings, offering multifunctional properties such as low electrical impedance, better mechanical properties, lower thickness, and on-demand controlled release of bioactive agents.

Methodology:
To this end, a conductive hydrogel with semi-interpenetrating polymer network (semi-IPN) structure comprised of temperature-responsive poly(N-isopropyl acrylamide) (PNIPAAm)-based copolymer and polythiophene (PT) was synthesized in this study and miniaturized via a nanofabrication method to be used as a neural interface.

Results:
The electrospinability of the solution was facilitated by the high molecular weight of the synthesized PNIPAAm-based block copolymer and its narrow molecular weight distribution. A cytocompatible and degradable dendrimer was used as the crosslinking agent of the semi-IPN with ample surface groups, which allowed a dual-hardening physical and chemical gelation process. Consequently, a lowered curing temperature was necessary to attain structural robustness at molecular and macroscopic levels. The copolymerization process reduced the volume phase transition temperature (VPTT) of pure PNIPAAm, and the resulted block copolymer showed lower overall transition energy. The fibrous hydrogel gave water molecules rapid access to the whole material and switched on a fast responsive characteristic. As the water impregnated the xerogel, the porosity and fiber diameter increased substantially. The developed material showed fast swelling and de-swelling responses triggered by temperature changes. Repeated hydration/dehydration cycles did not affect the physical integrity of produced electrospun fibers.
Furthermore, the conductive fibrous semi-IPN displayed a high electrical conductivity and charge storage capacitance compared to the conductive bulk hydrogel. This occurrence was

attributed to the formation of a large electrochemical surface area that resulted from system miniaturization. The impedance of the developed material was in the range of physiologically relevant frequencies.

Conclusion:
The incorporation of PT chains in the stimuli-responsive hydrogel network promoted the synergetic effect between the two components leading to the fabrication of a superior fibrous interpenetrating network neural interface with remarkable electrochemical properties.
Acknowledgment:
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keywords: Conductive, Hydrogel, Semi-IPN, System Miniaturization, Neural interfaces

83767228888

3D BIOPRINTED CONSTRUCTS TO GENERATE MATURE ORGANOIDS FROM IPSC-DERIVED RENAL PROGENITORS

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Chronic kidney disease (CKD) is characterised by the gradual loss of renal function. It affects approximately 10% of the population worldwide, and the only treatment is aimed to slow down the progression of kidney damage. Ultimately, CKD results in end-stage renal disease (ESRD), which can only be treated with dialysis or renal transplant. However, both options are far from ideal and cannot be considered permanent solutions.
Regenerative medicine, particularly the use of organoids, might provide a solution to this problem. Organoids are a relatively easy and scalable model that can be used to study organ development, regeneration, genetic diseases, and perform drug screening. However, the limited ability to accurately replicate adult organs’ maturation level, complexity, and functions drastically restrict their application in research and clinical medicine.We implemented differentiation protocols to obtain iPSC-derived metanephric mesenchyme (MM) and ureteric bud (UB) progenitors in sufficient numbers for bioprinting. We used a microfluidic 3D bioprinter capable of extruding core-shell filaments to manufacture renal constructs containing single cell progenitors. After bioprinting, we cultured the construct with an optimised mix of growth factors for two weeks. The 3D bioprinted renal progenitors showed high viability after bioprinting. After one day in culture, the cells self-aggregated into spheroids inside the hydrogel filaments. Within one week, renal vesicles were visible. Tubular structures were observed two weeks post-bioprinting, which stained positive for lotus tetragonolobus lectin (LTL) and e-cadherin. For the first time, we were able to bioprint iPSC-derived renal progenitors that generated renal organoids inside the bioprinted hydrogel constructs.

keywords: iPSC, Kidney organoids, 3D bioprinting, In vitro model

20941849987

A BIOFABRICATION TECHNOLOGY FOR GENERATING MULTISCALE CHANNELS IN HYDROGELS FOR COMPLEX 3D IN VITRO CO-CULTURES

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Native tissues are characterized by its 3D organization and distribution of cells, with specific cell-cell and cell-extracellular matrix (ECM) interactions dictating tissue function. The spatial distribution of cells and ECM in tissues is not arbitrary. There are specifically located cell populations for generating interconnected lumen structures, creating a fundamental structure-function relationship that determines the role of numerous tissues. Therefore, the capability to mimic this 3D environment is key for a correct in vitro modeling of tissues and for future tissue engineering applications.

Here we present a templating strategy using a thermally responsive polymer and we show the fabrication, in one step, of a network of interconnected channels within a hydrogel. While other polymers have been previously described for similar applications, we uniquely show that our template can create a defined 3D polymer scaffold to which cells can adhere, leading to the subsequent formation of a channel network that directly incorporates cells. This approach is based on a family of oxazoline polymers (POXA) that have been specifically designed to have a uniquely tunable range of lower critical solubility temperature (LCST), above which it remains insoluble and below which it is triggered to dissolve.

We were able to produce fibers with our POXA polymer with diameters as small as 5µm, up to the millimeter scale using melt electrowriting (MEW). We recorded stereomicroscope time lapses of their dissolutions while decreasing the temperature in a controlled way from 37°C to 4°C. Additionally, we monitored its water contact angle at different temperatures, confirming their change in stability and solubility. By using a customized fabrication method, we could include microchannels in a set of hydrogels with different crosslinking mechanisms: collagen, PEGDA, polyacrylamide and fibrin. Additionally, we were able to culture Schwann cells (SC) and HUVECs on top of these micro-scale fibers and transfer them to a hydrogel where the polymer would be dissolved, leaving cells growing in specific patterns inside of microchannels. With our method, we could fabricate in vitro models for vasculature by culturing HUVECs on the lumen of the channels and other cell types such as fibroblasts embedded in the surroundings. Allowing us to control the arrangement of the vascular channel as well as their interactions with the environment. We also showed how SC could migrate along these microchannels, as they would do in vivo, this is a key mechanism during tissue innervation, guiding axonal growth towards other tissues. Similarly, we cultured sensory neurons (nociceptors) derived from human iPSCs and showed how their axons could grow along the microchannels. Potentially becoming a new tool for nerve repair and tissue innervation studies or drug testing in clinical applications. Here, we demonstrate the value and versatility of this novel templating technology to generate complex 3D cell culture models. We believe that this system will allow the tissue engineering

field to fabricate more realistic in vitro models considering cellular arrangement and EMC interactions.

keywords: hydrogel, microchannels, POXA, in vitro

41883640155

MULTIMATERIAL COMPLEX TISSUE MODELS VIA SUSPENSION MEDIA-ENHANCED VOLUMETRIC BIOPRINTING

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INTRODUCTION: Major challenges in bioprinting tissues with functional, native-like behavior revolve around enabling the use of hydrogels with low elastic modulus, while also ensuring high shape fidelity and printing resolution. Such materials are necessary to allow cells to migrate, and to facilitate intercellular communication and reorganization of the neo-synthesized extracellular matrix. In this perspective, suspended bath bioprinting was previously developed as a printing technique that solves this problem by extruding bioinks within a yield-stress support bath that keeps bioinks with low viscosity in place until cured. Moreover, in order to increase the printing speed and overcome the geometric constraints of conventional layer-by-layer AM approaches, volumetric bioprinting was recently developed as a new light-based approach. However, the possibility to create high resolution features comprising multiple, independent structural elements intertwined into a single construct remains a major challenge, especially when using multiple materials and cell types in a single printing process. The current study describes a new biofabrication strategy that synergizes the multimaterial printing ability of extrusion in suspension media, and the layerless 3D patterning provided by visible-light tomographic printing, in order to rapidly fabricate complex tissue models with tunable mechanical properties, while embedding different cells types.

METHODOLOGY: A novel photo-crosslinkable bioresin was designed and characterized, based on Gelatin-Methacryloyl (GelMA) hydrogel microparticles that act like a Bingham plastic. This bioresin was used both as a support bath to enable deposition of soft hydrogels, and subsequently sculpted into a desired architecture via volumetric bioprinting, to leverage the microporosity provided by the packing of the microgels for cell infiltration and nutrient diffusion. In addition, two (bio)inks for the extrusion process were designed and mechanically characterized: i) a gellan gum (GG) and Poly(ethylene glycol) diacrylate (PEGDA) blend, used to create different mechanically-competent, reinforcing scaffolds within the volumetrically crosslinked GelMA microgels, ii) a blend of methylcellulose and fibrinogen, used as medium for cell printing.

RESULTS: Features smaller than 500 µm can be volumetrically printed in less than a minute with the microgel-bioresin, and 300 µm width filaments can be extruded within it with both bioinks. With a compression modulus ranging between 3 and 4 kPa, microgel-based samples have shown lower mechanical properties than bulk GelMA gels, but these could be enhanced and tuned using the GG/PEGDA ink. Printed reinforcing GGPEGDA/GelMA meshes taking up a 2.5% volume fraction of the whole slurry-based construct lead to increasing the compression modulus of

the composite by 40%. Printing of multiple cell types including vasculature forming endothelial cells and pancreatic cells was finally investigated to build complex biofabricated constructs for vascularized tissue engineering.

CONCLUSIONS: Combining extrusion-based bioprinting in a suspension media and volumetric bioprinting is an advantageous approach that allows to create complex cm3-scale and vascularized structures in a fast and accurate process, combining different biomaterials to tune both mechanical and biological characteristics. These features are crucial to better mimic the heterogeneous characteristics of living tissues (e.g., the complex architecture of the trabecular bone and the bone marrow, or the endocrine pancreatic tissue within the exocrine one).

keywords: Biofabrication, Tissue engineering, Bioprinting, Vascularization, 3D models

73296308379

KIDNEY-ON-A-CHIP - INTEGRATING GLOMERULAR FILTRATION AND TUBULAR REABSORPTION MODELS

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Introduction
The kidney plays a crucial role in drug development, as it dictates drug clearance and is a target for drug-induced toxicity. Nephrotoxicity of candidate drugs is one of the major reasons for drug attrition during preclinical, clinical and post-approval stages of drug development. These failures during the final stages of the drug development process are partially caused by the use of inaccurate preclinical nephrotoxicity models. Therefore, an accurate kidney model for Multi-Organ-Chip applications could revolutionize drug trials by providing a relevant in vitro platform.

Methodology
In this study, we generate an autologous kidney-on-a-chip that encompasses a glomerular and a tubular model. Induced pluripotent stem (iPS) cell-derived podocytes and tubular cells are seeded into the HUMIMIC Chip4, which enables the long-term co-cultivation of the renal model with up to three additional organ equivalents with a defined fluid flow and shear stress. The final maturation of the iPS cell-derived podocytes and tubular cells occurs within the Multi-Organ-Chip. After the renal cells’ final maturation, the co-culture can be maintained for at least 14 days.

Results
The kidney-on-a-chip exhibits a stable metabolism, a cellular barrier that prevents albumin from entering the excretory circuit, and the cells demonstrate a steady expression of key podocyte and tubular markers.

Conclusion
The kidney-on-a-chip can be employed for elaborate safety, efficacy and nephrotoxicity studies, as wells as for mechanistic studies of renal development or disease. The combination of the renal model with other organ equivalents further enables systemic studies, including ADME experiments. Therefore, the kidney-on-a-chip presents a human and systemic alternative to current in vivo and in vitro models.

keywords: kidney-on-a-chip, microphysiological system, iPSC

S07-1
Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration
Room: S3 A
(28 Jun 2022, 11:00 - 12:30)

Conveners:
Valeria Chiono; Michael Monaghan

20967802055

LESSONS LEARNED ON HOW (NOT TO) BUILD A HEART

Francesco Pasqualini (University of Pavia, Pavia, Italy)

The plan for tissue engineering has always been to deliver human tissue products that can repair, regenerate, and replace our organs. As far as the heart is concerned, that plan has been punched - hard - by reality (Cit. Mike Tyson). Through the lens of my post-doctoral research at Harvard, we will review advancements in heart muscle engineering and examine some of the fundamental roadblocks to its clinical translation. Specifically, we will see how simple bioengineering and image analysis tools can be used to establish unbiased and quantitative metrics of the phenotypic quality of stem cell-derived cardiac muscle cells. Further, we will see how techniques from biophysics can be used to provide a multiscale assessment of muscle contractility. Finally, we will examine how these tools can be expanded and leveraged into a new framework for cardiac tissue engineering where we stop "trying to build" a heart and we start "growing" one, instead. Round 2 with reality: ready, go!

keywords: stem cells, bioengineering, biophysics, cardiac, quality control and assurance

20967803906

MECHANICAL AND TOPOLOGICAL CUES TO ENHANCE DE NOVO EXTRACELLULAR MATRIX ELABORATION IN ELASTOMERIC SCAFFOLD MODELS.

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Elastomeric biodegradable scaffolds have been utilized as viable cardiovascular tissue surrogates in various applications, including cardiac patches, engineered vascular grafts, and heart valves. The interactions between cells and scaffold constitute an essential element in endogenous tissue growth and de novo tissue formation. Mechanical conditioning regimens are widely recognized as effective methods for facilitating extracellular matrix (ECM) accretion and improving engineered construct mechanical properties. Despite these advantageous factors, the understanding of the underlying cells - matrix interaction mechanisms remains relatively limited, hampering the development of in silico and in vitro models and the translation of engineered tissues into clinical application. In an attempt to reduce this gap in knowledge, we investigated how mechanical strain and micro-architecture impact ECM formation and elaboration. Vascular smooth muscle cells (VSMCs) have been micro-integrated into elastomeric biodegradable polyurethane scaffolds having identical microstructure. The constructs have been dynamically conditioned using a uniaxial stretch bioreactor for 21 days. Different levels of uniaxial strain, 15, 30, and 50%, have been continuously imposed at 1Hz of frequency for the entire culture period. We hypothesized that specific levels of strain and micro-architectures could be identified to enhance ECM production in quantity (collagen mass) and quality (anisotropy, stiffness). Samples were processed to evaluate ECM biosynthesis via: biochemical assay, qualitative and quantitative histological assessment, multi-photon analysis, and mechanical characterization. Experimental evaluation was coupled to a numerical model that elucidated the relationship between the scaffold micro-architecture and the strain acting on the cells. Results showed that while a 30% peak of strain level achieved maximum ECM synthesis rate, further increases in strain level led to a reduction in ECM. Likewise, micro-integrated scaffolds fabricated with different micro-architecture (i.e., different number of fibers intersections/area) have been exposed to 21 days of dynamic culture at a fixed 30% strain and a frequency of 1 Hz. Results highlighted the existence of specific micro-architectures and how topological cues are able to maximize ECM elaboration given a specific imposed macroscopic mechanical load. The improved understanding of the complex process of ECM formation in these mechanosensitive cell-scaffold models might lead to a more effective engineering and processing of cardiovascular tissue surrogates that are requested to function in highly demanding mechanical in-vivo environments.

keywords: Extracellular matrix, cardiovascular tissue, mechanical conditioning

20941820555

CONVERGENCY OF DUAL EXTRUSION BIOPRINTING AND MELT ELECTROWRITING ALLOWS FOR VASCULARIZED CARDIAC PATCH FABRICATION

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Introduction
Realization of tissue engineered cardiac constructs has progressed with the combination of induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and additive manufactured frameworks for guided repair[1]. Recently, we developed hexagonal 3D microfiber scaffolds with melt electrowriting (MEW). These scaffolds support contracting hiPSC-CMs and promote tissue organization and maturation[2]. However, scaling such constructs includes challenges, such as the need for nutrient and oxygen supply. We hypothesized that control over vascular patterning and deposition of cardiac cells can promote cardiac construct vascularization and native-like tissue formation. This study converges extrusion-based bioprinting and MEW to biofabricate the first anatomically-designed vascularized cardiac patch.

Methodology
Hexagonal MEW meshes were fabricated with a 3DDiscovery (REGENHU)[2]. Cell composition for myocardial and vascular components were tested using varied ratios of hiPSC-CMs, human fetal cardiac fibroblasts and human umbilical vein endothelial cells. Bioinks (myocardial and vascular) comprised of photo-crosslinkable GelMA with the addition of collagen or matrigel were optimized for both cellular performance and bioprintability by use of soluble fraction analysis, immunofluorescent staining, beating rate analysis and qPCR assessment. Viability after bioprinting was assessed using a metabolic assay and live/dead staining, comparing bioprinted to cast constructs. Finally, the myocardial and vascular bioinks were co-printed into the microfibers meshes using an anatomical design, modelled from a branch of the left anterior descending artery. The constructs were analyzed using immunofluorescent stainings.

Results
The myocardial bioink revealed enhanced native-like tissue formation using 6x107 cells/mL with a ratio of 9:1 (cardiomyocytes:fibroblasts). The vascular bioink enabled capillary-like network formations using 3x107 cells/mL with a ratio of 5:1 (endothelial cells:fibroblasts). Bioink optimization revealed that a combination of 5% GelMA with 0.8 mg/mL collagen, photocrosslinked using 0.25/2.5 mM (myocardial) and 0.5/5 mM (vascular) ruthenium/sodium persulfate had an optimal soluble graction and resulting cellular organization for both bioinks. The myocardial bioink exhibited a slowed, more synchronous beating rate pattern following 3 weeks in culture, indicating hiPSC-CM maturation. In addition, the formation of organized tissue-like structures was observed with an enhancement of troponin-T staining, compared to the other hydrogels tested. Bioprinting processes affected the vascular bioink resulting in increased metabolic output, as well as proliferation of the cells. The myocardial bioink showed a significantly reduced metabolic output 1 week following the bioprinting, however no noticeable difference in viability (live/dead assay) was observed between the bioprinted and cast groups.

Converged bioprinting into the MEW mesh was achieved by ensuring the gel viscosity that allowed for integration into the hexagonal pores with all pores filled after 2 printed layers. Interfaces between the vascular and myocardial patterned components were visualized using immunofluorescent morphological stainings.

Conclusions
This study has demonstrated potential for patterning anatomically-relevant vascular pathways within a bioprinted myocardial construct, forging an opportunity for scaling up tissue engineered constructs. Our study provides an important step towards the generation of 3D in vitro cardiac models of relevant dimensions with native-like tissue architecture and function.

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keywords: myocardium, vascularization, bioprinting, tissue engineering, bioink

20941815786

ALLOGENEIC STEM CELLS AND IMMUNOMODULATORY BIOMATERIALS FOR CARDIAC TISSUE ENGINEERING

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Introduction: Stem cells are being tested in clinical trials for cardiac repair. Bone marrow derived allogeneic (unrelated donor) mesenchymal stem cells and induced pluripotent stem cells (iPSC) have emerged as ideal cell types for cardiac repair and regeneration. Outcome of initial allogeneic stem cells based clinical trials was positive. There were no significant side effects observed after cell transplantation. In fact, the implanted cells were able to improve cardiac function. However, poor survival of transplanted cells in the myocardium is a major hurdle in clinical translation of stem cell-based therapies for cardiac repair.

Methods: We have previously reported that after transplantation in the ischemic heart stem cells display immunogenicity and are rejected by recipient immune system. In our ongoing studies, we have been engaged in developing immunomodulatory biomaterials-based strategies to prolong survival of transplanted stem cells in the heart. We synthesized MXene quantum dots (MQDs) with tailored surface properties to possess intrinsic immunomodulatory properties.

Results and conclusion: Our data demonstrate that MQDs were spontaneously uptaken into antigen-presenting cells and downregulated the expression of genes involved in alloantigen presentation, and consequently reduced the activation of allogeneic lymphocytes. Furthermore, MQDs are able to selectively reduce activation of CD4+IFN-γ+ T-lymphocytes and promote expansion of immunosuppressive CD4+CD25+FoxP3+ regulatory T-cells in a stimulated human lymphocyte population. Furthermore, MQDs are biocompatible with MSCs and iPSC. Next, MQDs were incorporated into a chitosan-based hydrogel to create a 3D platform for stem cell delivery to the heart. This composite immunomodulatory hydrogel-based platform improved survival of stem cells and mitigated allo-immune responses. These findings suggest that this new class of biomaterials may bridge the translational gap in stem cells and biomaterials-based strategies for cardiac tissue engineering.

keywords: Stem Cells; Biomaterials; Cardiac Tissue Engineering

94238124605

A MICRO-PRECISION ELECTRO ARRAY (MPEA) PLATFORM INTEGRATED WITHIN A MECHANICALLY ACTIVE HEART-ON-CHIP FOR MODELLING DILATED CARDIOMYOPATHY

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Modeling the cardiac pathological traits would be of paramount utility to elucidate possible targets of new therapeutics for unmet pathology (e.g., Dilated Cardiomyopathy (DCM [1])). Traditional in vitro systems lack the complexity of human physiological conditions, resulting poorly reliable for tissue engineering studies. Organs-on-chip (OoC) have been shown to be a promising alternative, offering the ease to integrate stimulation and sensors [2]. In previously developed beating heart-on-chip [3]–[5], despite advanced tissue maturation through mechanical stimulation have been demonstrated, the coupling with electrical readout allowing for a broader electrophysiological recording space and high throughput analyses is still missing. To overcome these issues, we developed µPEA, an electrode array system coupled with the beating-heart-on-chip [5] able to provide 3D microtissues with mechanical stimulation and electrical readouts, using noninvasive 2D electrodes. We validated µPEA-Heart-on-Chip by assessing electrophysiology of neonatal rat cells and we exploited it to study the effect of mechanical stimulation on human DCM models.

Methodology:
Electrodes were designed using CAD and developed in glass substrate using physical vapor deposition (Cr/Au, 100nm). Electrodes arrays consist of five recordings, two references and one ground electrodes for measuring field potential. Chips were connected to a platform interface with a multiplexing system followed by an amplifier coupled with a bandpass filter (0.5-100Hz). Electrodes characterizations were done by measuring impedance spectroscopy and by filling the OoC with PBS. iPSC-CM were generated by following well-established differentiation protocols of iPSC derived from DCM patients [6]. Neonatal rat cardiomyocytes, DCM iPSC-CMs, and isogenic controls were embedded in fibrin gel at 80-120 · 106 cells/mL and cultured for 5-10 days in static or mechanically active (i.e. 10% uniaxial strain at 1Hz) environment. After maturation, electrophysiological signals were measured from different electrodes.

Results:
The µPEA-Heart-on-Chip was successfully assembled by integrating and aligning the innovative patterned surface with the mechanically active OoC (the electrodes are positioned along the cardiac microtissue). Electrodes impedance measurements were 124.5 ± 3.1 KΩ at 1Khz, in line with literature values [7]. Neonatal rat cardiomyocytes microtissues started to spontaneously beat after 4 days in culture. Field potentials were successfully measured through all five different electrodes and key parameters were evaluated. DCM iPSC-CM were efficiently differentiated in 2D, as evidenced by immunofluorescence staining of cardiac Troponin T. The DCM diseased model was established within the µPEA-Heart-on-Chip, with cells that organized and interconnected within the 3D environment. Electrophysiological and contractility changes of the DCM model in response to the mechanical stimulation is currently under evaluation.

In Conclusion, here we described the development of the µPEA-Heart-on-Chip capable to

integrate a mechanical stimulation with an electrophysiological activity recording system. The platform represents an unprecedented tool to investigate electrical cardiac alterations in healthy or diseased model in response to mechanical stimulation.

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keywords: Cardiomyopathy, electrophysiology, heart-on-chip

62825449926

CARDIAC TISSUE-LIKE 3D MICROENVIRONMENT ENHANCES THE DIRECT REPROGRAMMING PATH OF HUMAN FIBROBLASTS INTO INDUCED CARDIOMYOCYTES BY MICRORNAS

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Restoration of cardiac functionality after myocardial infarction represents a major clinical challenge1. Recently, we found that transient transfection with a microRNA combination (miRcombo: miR-1, miR-133, miR-208 and 499) is able to trigger direct reprogramming of adult human cardiac fibroblasts (AHCFs) into induced cardiomyocyte (iCMs) in vitro2. However, achieving efficient direct reprogramming still remains a challenge. Direct reprogramming of human fibroblasts could be enhanced by culturing miRcombo-transfected cells in a three-dimensional (3D) environment with biomimetic biophysical and biochemical cues3,4. Herein, the ability of cardiac-like extracellular matrix (called Biomatrix, BM) to enhance miRcombo-mediated direct cell reprogramming was studied. Then, miRcombo-transfected cells were cultured in a biomimetic microenvironment consisting of a 3D fibrin hydrogel containing BM.

Methodology
BM was produced by in vitro culture of AHCFs for 21 days, followed by decellularization. AHCFs were transfected with miRcombo and then cultured for 2 weeks on the surface of: uncoated and BM-coated polystyrene (PS) dishes, on the top of fibrin hydrogels (2D hydrogel), or embedded into 3D fibrin hydrogels without (3D hydrogel) or with BM (3D BM hydrogels). The expression of cardiac markers and cell maturation was analysed by ddPCR, immunofluorescence and calcium transient analysis.

Results
Culture of miRcombo-transfected cells on BM-coated vs. uncoated PS dishes enhanced direct reprogramming efficiency, enhancing TNNT2, ACTC1 and CACNA1C expression and increasing the percentage of cardiac troponin T (cTnT)-positive cells at 15 days culture time. Culture in 3D hydrogel after miRcombo transfection significantly improved direct reprogramming efficiency respect to uncoated PS and 2D hydrogel, increasing TNNT2, SCN5A and MYL7 expression. Finally, miRcombo-transfected cells were cultured in 3D BM hydrogel providing cardiac tissue-mimetic biophysical and biochemical cues. The expressions of cardiomyocyte genes and cTnT were significantly enhanced in cells cultured for 15 days in 3D BM compared to 3D hydrogels. Calcium transient was enhanced in 3D BM compared to 3D hydrogels in miRcombo cells, showing higher slope of calcium upstroke.

Conclusions
Overall results demonstrated that a biomimetic 3D culture microenvironment can enhance the direct reprogramming efficiency of miRcombo-transfected human adult cardiac fibroblasts into

iCMs. Future investigations will elucidate which molecular barriers to direct reprogramming can be overcome by the use of 3D biomimetic cell culture substrates, paving the way to the research for more efficient strategies for direct cardiac reprogramming.

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keywords: microRNAs, direct reprogramming, three-dimensional culture, cardiac ECM Introduction

41883671128

TISSUE ENGINEERED CARDIAC PATCHES FOR THE TREATMENT OF POST-MI HEART FAILURE USING NATURAL POLYMERS AND HUMAN IPSC-DERIVED CELLS

Annabelle Fricker (University of Sheffield, Sheffield, United Kingdom), David Gregory (University of Sheffield, Sheffield, United Kingdom), Sian Harding (Imperial College London, London, United Kingdom), Ipsita Roy (University of Sheffield, Sheffield, United Kingdom)

INTRODUCTION: Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, contributing a huge burden on healthcare providers. Myocardial infarction (MI) is one of the most fatal results of CVDs as it can lead to ultimate heart failure. Available treatments are used to mitigate many of the symptoms of MI, however they are not designed to repair the damaged tissue. A proposed solution to this lack of a regenerative treatment is a tissue engineered myocardial patch which would deliver healthy cells to repopulate the infarct area. In this research, natural and biocompatible materials, a polyhydroxyalkanoate (PHA)1-3 and alginate3, are used with the aim of producing a cellular multimaterial myocardial patch for this purpose. The patch would incorporate human induced pluripotent cardiomyocytes (hiPSC-CMs) and endothelial cells (hiPSC-ECs)

METHODS: Bacterial fermentation of Pseudomonas species was carried out to produce the PHA poly(3-hydroxyocatnoate-co-3-hydroxydecanoate), P(3HO-co-3HD), and this was purified and extracted from the bacteria using Soxhlet extraction. Resazurin assays with a C2C12 myoblast cell line were used to test the biocompatibility of P(3HO-co-3HD) and alginate hydrogel, the polymers were 3D printed (fused deposition modelling) to produce a multimaterial patch, with C2C12 cells encapsulated in the alginate and 3D-bioprinted. hiPSC-CMs and hiPSC-ECs were produced from (hiPSCs), seeded onto P(3HO-co-3HD) films, live/dead stained, and functionally analysed. Multimaterial patches were also tested in vivo in a rat model with an induced infarct.

RESULTS: P(3HO-co-3HD) has been successfully produced and characterised to confirm its chemical structure and 3HO:3HD molar ratio; mechanical properties which show that it is highly elastomeric, making it a suitable polymer for myocardial applications; and thermal properties, including a melting point of around 54oC making it easy to 3D print. P(3HO-co-3HD) and alginate were shown to both be non-cytotoxic via the resazurin assay. 3D printing was carried out with these materials, with C2C12 cells encapsulated in the alginate, and successfully produced cellular multimaterial patches at a high resolution, while maintaining cell viability. To improve the cell types included in the patch, hiPSC-CMs and hiPSC-ECs were produced, with initial results showing that they adhere to P(3HO-co-3HD) with good viability, and retain functionality through beating and calcium handling, as seen with Fluo-4 imaging.

CONCLUSIONS: Multimaterial patch production and successful encapsulation and printing of cells shows promise for the development of a functional cellular multimaterial patch. Future aims in this project are to include hiPSC-ECs and hiPSC-CMs in the multimaterial patch before carrying out in vitro and in vivo experiments in a rodent MI model.

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keywords: Cardiovascular, iPSCs, 3D printing, Biomaterials

S07-2
Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration
Room: S3 A
(28 Jun 2022, 15:30 - 17:00)

Conveners:
Valeria Chiono; Michael Monaghan

62825434605

INJECTABLE HYDROGEL FOR MICRORNA RELEASE IN CARDIAC REGENERATIVE MEDICINE

Letizia Nicoletti (POLITECNICO DI TORINO, TORINO, Italy), Elena Marcello (POLITECNICO DI TORINO, TORINO, Italy), Camilla Paoletti (POLITECNICO DI TORINO, TORINO, Italy), Barbara Stella (Department of Drug Science and Technology, TORINO, Italy), Silvia Arpicco (Department of Drug Science and Technology, TORINO, Italy), Clara Mattu (POLITECNICO DI TORINO, TORINO, Italy), Valeria Chiono (POLITECNICO DI TORINO, TORINO, Italy)

INTRODUCTION
Myocardial infarction (MI) leads to a significant loss of cardiomyocytes followed by the progressive formation of a non-contractile fibrotic scar. Recently, the use of microRNA (miRNA) has emerged as a promising strategy for cardiac regeneration. Paoletti et al. demonstrated that the transient transfection with four microRNA mimics (termed “miRcombo”) can induce the direct reprogramming of human cardiac fibroblasts (CF) into induced cardiomyocytes (iCMs)[1]. To overcome the limitations of in vivo miRNAs administration, proper nanocarriers are required. Moreover, alginate-based injectable hydrogels can be exploited for controlled in situ release of miRNAs-loaded nanocarriers [2]. However, alginate presents some limitations such as low degradability in vivo and limited cell adhesion.
In this work, new lipoplexes (LPs) loaded with miRcombo were developed to obtain more efficient encapsulation and release of miRcombo to CFs compared to a commercial agent. Then, LPs were combined with an optimized alginate dialdehyde (ADA) hydrogel for potential in situ controlled release of miRNA in the damaged tissue.

METHODS
LPs containing negmiR (negative control) or miRcombo based on a mixture of cationic and helper lipid were prepared at different N/P ratio (N/P 3 - 0.35) and physiochemically characterized [3]. Adult human CFs (AHCFs) were treated with miRcombo-loaded LPs to assess transfection efficiency and ability to promote direct reprogramming of AHCFs into iCMs through gene expression analysis at 15 days culture time. ADA was prepared by ALG oxidation using sodium metaperiodate [2]. ADA-based hydrogels with different compositions were characterised for their physicochemical properties. Preliminarily, siRNA-Cy5-loaded LPs were encapsulated into ADA-based hydrogels with selected composition and Cy5-siRNA release was studied.

RESULTS
MiRcombo-loaded LPs with optimal N/P ratio of 3 were selected based on stability studies (evaluated in different media by DLS analysis), showing high encapsulation efficiency (99%). In vitro cell test of LPs with AHCFs showed high biocompatibility and miRNA cellular uptake. Moreover, treatment of AHCFs with miRcombo-loaded LPs favoured their direct reprogramming into cardiomyocyte-like cells, evaluated through the expression of cardiomyocyte markers such as cardiac troponin C (cTnT).
ADA was prepared with an average yield of 68% and an oxidation degree of 23%. ADA concentration and ionic crosslinking were optimised to develop injectable hydrogels with cardiac-like viscoelastic properties. Model Cy5-siRNA-loaded LPs were physically entrapped within ADA-based hydrogels, completely releasing Cy5-siRNA within 24h.

CONCLUSION
A novel miRNA-delivery system, consisting of miRNA-loaded LPs encapsulated in an ADA-based injectable hydrogel, was developed for cardiac regenerative medicine. Cy5-siRNA release data

suggested the need for tailoring the surface charge of miRNA-loaded LPs by proper coating, to minimize the electrostatic interactions between ADA and the positively charged miRNA-loaded LPs. This activity is currently in progress and preliminary data showed an enhancement in the stability of hydrogel-embedded LPs by tailoring their surface charge.

1 Paoletti C, et al. Front Bioeng Biotechnol. ,8,529 (2020). 2 . Sarker, B et al., J. Mater. Chem. B, 2(11), 1470-1482 (2014). 3. Nicoletti et al., Nanomed.: Nanotechnol. Biol. Med., under submission.
BIORECAR is supported from the European Research Council (ERC) under the EU H2020 research and innovation programme (GA N° 772168).

keywords: Lipoplexes, miRNA, alginate, hydrogel, cardiac regeneration

52354564359

BIOFABRICATION OF SCAFFOLD-FREE 3D CELLULAR STRUCTURES USING MAGNETIC LEVITATIONAL ASSEMBLY TO STUDY CARDIAC TOXICITY

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Spheroids are one of the well-characterized 3D cell culture approaches for drug screening and therapeutic studies. Magnetic levitation (MagLev) is a newly developing approach to form 3D cellular structures and spheroids [1,2,3]. Magnetic levitational assembly of cells provides rapid, simple, cost-effective 3D cell culture formation while ensuring scaffold-free microenvironment. Here, our efforts are summarized in designing new magnetic levitation platform and biofabrication of 3D cellular entities via magnetic levitation for tissue engineering. Magnetic levitation and guidance of cells were provided by using a paramagnetic agent to fabricate scaffold-free 3D cellular structures. The parameters of cell density, paramagnetic agent concentration, and culturing time were optimized to obtain 3D cardiac cellular structures with tunable size, circularity, and high cell viability. Cellular and extracellular components of the 3D cellular structures were demonstrated via immunofluorescent staining. Also, 3D cardiac cellular structures showed more resistance to drug exposure compared to 2D control. In conclusion, MagLev methodology offers an easy and efficient way to fabricate 3D cellular structures for drug screening studies.

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keywords: magnetic levitation, scaffold-free tissue engineering, 3D cell culture, drug screening

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AN INDUCED PLURIPOTENT STEM CELL-BASED MODEL TO STUDY THE MECHANOBIOLOGY OF MYOCARDIAL FIBROSIS

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Cardiac fibrosis is the consequence of chronic insults on the myocardium, and it is characterized by the abnormal accumulation of extracellular matrix (ECM). The transdifferentiation of cardiac fibroblasts (cFbs) into myofibroblasts drives pathological ECM remodeling, a process highlighted by biochemical and structural changes which compromise cardiomyocytes (CMs) contractile activity and eventually lead to heart failure [1]. Here, we adopted bioengineering tools and induced pluripotent stem cells (iPSCs) to investigate how fibrotic ECM affects the structural and functional properties of CMs.
Thus, we derived cFbs from induced pluripotent stem cells (iPSCs-cFbs) and optimized a protocol based on biochemical and mechanical stimulation to induce their transdifferentiation to myofibroblasts. Next, we obtained fibrotic ECM (dECM) by implementing a decellularization procedure of the activated iPSCs-cFbs and analyzed the pathological changes occurring during the deposition of cardiac diseased ECM. The results generated through this analysis were confirmed by studying dECM of cFbs isolated from heart failure patients and their healthy counterparts. Then, we generated iPSCs-CMs and cultured them either on healthy or fibrotic dECM. Morphological and functional analyses were implemented to study how the biomechanical properties of pathological ECM affect CMs physiology and function.
Finally, we established a 3D in vitro culture system, which entails the co-culture of isogenic iPSCs-CMs and -cFbs that better reproduces the cellular complexity and functionality of the human heart and represents a powerful tool for personalized medicine applications.
By capitalizing on this approach, we might be able to recapitulate the accumulation of fibrotic tissue occurring during heart disease and investigate the contribution of pathological ECM to the progression of heart failure.

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keywords: iPSCs; iPSCs-cFbs; ECM; dECM; iPSCs-CMs

52354501244

ELECTROCONDUCTIVE SCAFFOLDS FOR IN VITRO CARDIAC MODELS

Matteo Solazzo (Trinity College Dublin, Dublin, Ireland), Michael G. Monaghan (Trinity College Dublin, Dublin, Ireland)

Introduction
With continued progress of wearable sensor technology and drug-screening in-vitro models, there is a need for more advanced biomaterials and scaffolds to enhance electrical performance for stimulation and recording.1 Poly(3,4-ethylenedioxythiophene):poly-styrenesulfonate (PEDOT:PSS) is an electroconductive polymer often applied within biosensors and more recently as scaffold in tissue engineering.2 In this context, its conductive properties are hypothesized to enhance the effect of electrical stimulation; known to play a potent role in differentiation of progenitor stem cell sources into cardiomyocytes and in the maturation of cardiac engineered organoids.3
In this project, PEDOT:PSS was engineered into tunable, aligned, three-dimensional (3D) porous sponge-like structures. Scaffolds were functionalised via a crystallisation treatment to enhance their properties for tissue engineering. Afterward, we conceptualized and fabricated a bioelectric pacing bioreactor to deliver electrical stimulation to 3D scaffolds and an ad-hoc rig for in-vitro contraction-tracking, validated in-vitro using C3H10, primary rat cardiomyocytes and induced pluripotent stem cell derived cardiomyocytes.

Materials and methods
PEDOT:PSS was covalently crosslinked using glycidoxypropyl-trimethoxysilane (GOPS) and fabricated via lyophilisation. Crystallisation was achieved with incubation in pure sulphuric acid to improve conduction networks and remove excess PSS. Morphology of constructs was evaluated qualitatively using scanning electron microscopy (SEM) and quantitatively through image analysis of scaffold microtomed sections. Ethanol intrusion provided quantification of the overall porosity. Mechanical properties were determined using a Zwick-Roell uniaxial testing apparatus, while electrical features were simultaneously obtained from a Keithley sourcemeter. Via X-ray diffraction (XRD) it was possible to confirm the crystallisation of PEDOT:PSS. In-vitro studies determined the material biocompatibility and effectiveness of custom designed bioreactor. Viability, proliferation via DNA quantitation, metabolism and cell orientation were chosen as performance indicators. Bioreactor designs were generated with Solidworks® and rapid-prototyped with either Prusa-i3 or Formlabs SLA printers. Matlab® was adopted for the writing of scripts and the analysis of datasets, such as piezoresistivity, pore size, stress-relaxation, cell-directionality.

Results & Discussion
Controlled freeze-drying parameters achieved highly porous structures with either isotropic or aligned architectures. Crystallised scaffolds exhibited 1000-fold higher conductivity compared to untreated ones, while preserving stiffness and biocompatibility in a range matching to induce myogenic differentiation.4 We designed and prototyped both a pacing bioreactor that can fit standard 6-well plate and a chip with flexible anchorage for tracking of contraction (R3S), that is reusable and easy to manufacture. A 7-day study applying electrical pacing to C3H10 cells, showed that pacing does not decrease cells viability, and that it also promotes metabolism and alignment of cells, synergistically with the aligned topography of the scaffolds. Studies on primary rat cardiomyocytes and induced pluripotent stem cell derived cardiomyocytes further corroborated the use of these scaffolds for in vitro models.

Conclusion
Overall, PEDOT:PSS scaffolds provided an asset for the production of versatile platforms for tissue engineering applications.

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keywords: PEDOT:PSS, electroconductive scaffolds, in vitro model, cardiac tissue engineering

41883604207

HARNESSING THE POTENTIAL OF IMMUNE CELLS TO PROMOTE CARDIAC REPAIR FOLLOWING MYOCARDIAL INFARCTION

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Purpose: Each year, 15 million people suffer from a myocardial infarction and heart failure, resulting in one of the leading causes of death worldwide. Owing to the fact that the adult mammalian heart lacks a regenerative capacity, the ischemic cardiac muscle is replaced by scar tissue. While the mechanisms involved in fibrotic tissue formation are still elusive, the immune system is known to play a critical role. Therefore, modulating the immune response after cardiac injury is becoming a promising strategy to prevent scar formation and improve heart function after myocardial infarction.

Methods: Using a permanent left coronary artery ligation mouse model, we assessed the cardiac repair after myocardial infarction, in two conditions. The first condition involved depletion of a specific immune cell subtype with diphtheria toxin, using a genetically engineered model. On the other hand, the second condition involved the adoptive transfer of these cells in a clinically relevant manner, one day post ischemia, in wild type mice. The reparative cardiac capacity was assessed with echocardiography four weeks post ischemia, an angiogenesis assay, and Mason's trichrome staining to assess the fibrotic area. To investigate the mechanisms of cardiac repair, we performed an EdU proliferation assay and a TUNEL survival assay on cardiomyocytes. Moreover, we performed flow cytometry and RNA sequencing on various immune cell subsets isolated from the infarcts to determine changes in the inflammatory response.

Results: Upon depletion of this particular immune cell subset, there was a significant increase in infarct size and a reduced left ventricular contractility (reduced ejection fraction and increased end diastolic volume), as evidenced with echocardiography. On the other hand, the adoptive transfer of the immune cell subset improved cardiac repair by reducing fibrosis and increasing the left ventricular contractility. This functional improvement was accompanied by enhanced angiogenesis in the infarct area, reduced cardiomyocyte cell death and moderately increased cardiomyocyte proliferation. In addition, the immune cells delivered by adoptive transfer accumulated at the site of cardiac injury and in secondary lymphoid organs post myocardial infarction. Mechanistically the pro-repair effect of this therapy was attributed to the differences in accumulation and the inflammatory phenotype of T cells and macrophages in the infarcts.

Conclusion: Taken together, we found that the delivery of this immune cell subset post ischemia diminished scar tissue formation by acting on multiple target populations and cellular processes, resulting in long-term improvement of cardiac function. This study demonstrates the potential of using this immune cell subset as a therapy for patients with myocardial infarction and potential heart failure.

keywords: Immune cells, cardiac repair, myocardial infarction

94238132466

DESIGN AND FABRICATION OF ADVANCED THICK HUMAN CARDIAC ENGINEERED TISSUES

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Introduction
Cardiovascular diseases remain the leading cause of death worldwide. Our research focuses in building cardiac microtissues that resemble the native heart as closely as possible, in terms of both structure and function. Across its thickness, native myocardium is built of several thin tissue sheaths, and the cardiomyocytes (CMs) in each of the layers are aligned, and thus contract, in a specific direction. Herein we aim at mimicking the laminar architecture of the myocardium, by producing tissue engineered constructs which show a preferential direction of contraction, and can be assembled to achieve relevant tissue thickness.

Methodology
Fibrous scaffolds with a diamond pattern were manufactured by melt electrospinning writing (MEW) of medical grade polycaprolactone, mechanically characterized and subsequently seeded with a mixture of 90% CMs and 10% cardiac fibroblasts, both obtained from the differentiation of human induced pluripotent stem cells. Samples were kept in culture and fully characterized, and their beating compared to that of other pore geometries.

Results
Myocardial tissue from porcine samples was histologically characterized in order to determine the variation of the fiber orientation from epicardium to endocardium, showing an angle variation of 6.39°/mm. A design with diamond-shaped pores was predicted to produce an in-plane contraction. MEW proved to be a reproducible and accurate method for printing these scaffolds, which showed adequate levels of compliance when mechanically tested. Engineered cardiac microtissues exhibited relevant cardiac-like features, including beating rate, sarcomere length and gene expression, as well as good viability and metabolic activity. When compared to other pore geometries, diamond-patterned scaffolds not only contracted along a preferential direction we had anticipated (that of lower mechanical resistance, i.e., the short axis of the diamonds), but also displayed greater magnitude and velocity of contraction than squared and rectangular scaffolds. Furthermore, optical mapping of the constructs showed better electrophysiological properties for the diamond-patterned samples, with values closer to native human cardiac tissue. Individual constructs could be stacked to a total thickness of ≈800 μm, and showed good cohesion of the distinct layers as well as more complex beating patterns.

Conclusions
In this work we developed a diamond patterned, melt electrospun scaffold, and show how this particular architecture favours the biomimetic contraction of seeded CMs along the short axis of the diamonds. By subsequently stacking several scaffolds with distinctly oriented diamonds, we obtained cardiac microtissues with increased biological representativity, in terms of their thickness, their multi-layered structure, and the varying principal orientation of each of the layers. Constructs demonstrated an adequate performance in vitro and were also tested in vivo, and show great potential for cardiac tissue engineering and regenerative medicine applications.

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keywords: cardiac tissue engineering, melt electrospinning writing, biomimetic scaffolds, cardiomyocytes, cardiac regeneration

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BIOMECHANICALLY STIMULATED 3D ENDOTHELIAL GUT-ON-CHIP PLATFORM TO STUDY INTESTINE MICROBIOME AND IMMUNE SYSTEM INTERACTIONS

Karol Kugiejko (MiMic Lab, Department of Electronics, Information and Bioengineering, Politecnico di Milano; Department of Experimental Oncology, Istituto Europeo di Oncologia – IRCCS, Milan, Italy), Mattia Ballerini (MiMic Lab, Department of Electronics, Information and Bioengineering, Politecnico di Milano; Department of Experimental Oncology, Istituto Europeo di Oncologia – IRCCS, Milan, Italy), Luigi Nezi (Department of Experimental Oncology, Istituto Europeo di Oncologia – IRCCS, Milan, Italy), Marco Rasponi (MiMic Lab, Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy)

The human gut microbiota constitutes the most bountiful and divergent community of organisms compared to the other areas of the body [1]. Growing attention is devoted to the bacterial equilibrium and constitution in human intestines, being highly plastic over time [2,3]. Bacteria present a significant part in response to immunotherapy in cancer [4]. Meanwhile, predominant cell cultures and animal models encounter substantial limitations [5].

3D-multi-compartment microfluidic cultures may overcome met obstacles, mimicking elaborate multicellular architectures and niches, while maintaining high control. Among the numerous gut-on-chip designs, the differentiation between 2D and 3D cellular microenvironments is possible, either mechanically stimulated or not. Mechanobiological actuation and 3D niche recapitulation proved integral in maturing and translating models from in vivo to in vitro [6,7,8].

Here, we present the intestine-on-chip PDMS-based device with endothelium captured in 3D under mechanobiological stimulation. As a proof-of-concept, the device integrates actuation on a three-cell types coculture, which includes human micro-endothelial cells and two human intestinal epithelial cell lines endowed with different adsorbing and secretory properties, respectively. A collagen-based extracellular-matrix compartment connects the intestinal and the vascular spaces. Multi-parametric assessment of cell viability and function at different time points describes the influence of mechanobiological stimuli on the maturation of the gut endothelium. Transcriptional profiling of the epithelial cells and functional characterization of different primary immune cells illustrate the suitability of the system to dissect complex interactions between components of the tumour microenvironment.

The biomechanically stimulated 3D intestine-on-chip provides an elegant platform to study how microorganisms inflect the crosstalk between epithelial and endothelial compartments in the gut and portray a relevant alternative for preclinical studies. The acquired model allows dissecting the trans-endothelial migration of immune cells in health and disease.

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keywords: Organs-on-Chips, Gut-on-a-Chip, Intestine-on-a-Chip, Microfluidics

S08
Antimicrobial biomaterials for bone regeneration
Room: S4 A
(28 Jun 2022, 11:00 - 12:30)

Conveners:
Fergal O'Brien; Joanna Sadowska

62903406568

BIOACTIVE GLASS BASED APPROACHES FOR ANTIBACTERIAL BONE REGENERATION

Aldo Boccaccini (University of Erlangen-Nuremberg / Institute of Biomaterials, Erlangen, Germany)

Bioactive glasses (BGs) are being increasingly investigated as antibacterial materials for developing scaffolds for bone tissue engineering (TE). Such applications are based on the biochemical reactions occurring on the BG surface in contact with the biological environment, which involve the (controlled) release of biologically active ions during BG scaffold degradation. Such ions are capable of stimulating specific cellular responses involved in bone tissue growth [1]. In addition, BGs can induce immunomodulatory effects to trigger bone regeneration and wound healing [2]. Expressing a further functionality of BGs, specific compositions are designed to release ions with antibacterial effect (Ag, Cu, Zn, Ga among other).

In this presentation, ion doped BGs will be discussed in the broad context of their use in bone tissue engineering. In particular, results will be presented to illustrate the osteogenic, angiogenic and antibacterial effect of a series of silicate and borate BGs. Specific concentrations of such ions enhance the secretion of vascular endothelial growth factor and the different mechanisms by which different ions induce angiogenesis will be discussed. The results of vitro studies will be presented that show the effect of ion concentration (following BG dissolution) on stem cell behavior. In an attempt to enhance the mechanical and biological performance of BG scaffolds, the polymer coating approach will be discussed. Such coatings (using synthetic or natural polymers) provide additional functionalities [3], for example, incorporating mesoporous BG nanoparticles as therapeutic drug carriers. Thus, the dual, simultaneous and tuned release of biologically active ions and therapeutic drugs (both antibiotics and growth factors) from BG scaffolds can be exploited to induce synergistic antibacterial, osteogenic and angiogenic effects.

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keywords: bioactive glass, scaffolds, bone regeneration

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POLYHYDROXYALKANOATE/BIOACTIVE GLASS COMPOSITE SCAFFOLDS WITH ANTIMICROBIAL PROPERTIES FOR BONE TISSUE ENGINEERING APPLICATIONS

Andrea Mele (University of Sheffield, Sheffield, United Kingdom), Aldo R. Boccaccini (University of Erlangen-Nuremberg, Erlangen, Germany), Muhammad Atiq Ur Rehman (Institute of Space Technology, Islamabad, Pakistan), Ipsita Roy (University of Sheffield, Sheffield, United Kingdom)

Introduction
Bone tumour removal, traumas with large defects or infections, and degenerative diseases are the main catastrophic events impeding complete bone healing. Autologous and allogenic bone grafting, and biologically inert metallic devices have limitations such as non-availability of autogenous bone, risk of infectious disease transmission, subsequent surgical removal, and bacterial infections [1]. Therefore, to overcome the limitations, we fabricated composite scaffolds based on a combination of Poly(3-hydroxybutyrate) [P(3HB)], a natural biocompatible and bioresorbable polymer of bacterial origin, and a Borosilicate-based bioactive glass doped with Zinc (BS-Zn). Moreover, the Zinc-doped bioglass is used to provide antibacterial activity, as Zn2+ is known for its strong anti-inflammatory and bactericidal properties. [2]

Methodology
P(3HB) was produced by bacterial fermentation of B. sacchari in the presence of excess of carbon and nitrogen limitation. Gas chromatography-mass spectrometry (GC-MS), Fourier-transform Infrared spectroscopy (FTIR), Nuclear Magnetic Resonance spectroscopy (NMR), and Differential Scanning Calorimetry (DSC) have been conducted to characterise the resulting polymer. Solvent casting has been used to produce composite films and X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), High-resolution X-ray CT and tensile testing were conducted to determine their properties.

MG63 human osteoblast cell line was cultured on the composite and neat polymer films to assess biocompatibility, quantified using the resazurin assay. Subsequently, Live/Dead assay was performed using calcein green and ethidium bromide. In order to evaluate the antimicrobial activity, Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) were determined using ISO20776 against E. coli 8739, S. aureus 2569 and S. aureus 6538P for P(3HB)/BS films loaded with Gentamicin and P(3HB)/BS-Zn. The HALO Test was also carried out for the composite films.

Results
Chemical analysis confirmed that the polymer produced was Poly(3-hydroxybutyrate). The thermal properties of the extracted polymer analysed by DSC were found to be very similar to previous studies and commercially available products [3]. XRD analysis resulted in profiles which indicated the amorphous nature of the bioactive glasses. The addition of nanosized bioglasses induced a nanostructured topography on the surface of the composites, as visible by SEM images. Moreover, X-ray CT confirmed the homogeneous distribution of the bioglass filler in the polymeric matrix. The resazurin assay demonstrated the biocompatibility of P(3HB) and the related composites. The MIC/MBC test and the HALO test confirmed the antimicrobial activity of the composites.

Conclusions
In this study, a short-chain length PHA was produced by bacterial fermentation and its

properties were investigated. Composite films have been synthesised using Borosilicate-based bioactive glasses. These have been further characterised to confirm the properties of the composite materials. The biocompatibility of the films was confirmed using the MG63 human osteoblast cell line. Also, the antimicrobial activity of P(3HB)/BS composite films loaded with gentamicin and P(3HB)/BS-Zn were investigated. The final aim of this project is the development of a promising material with antimicrobial activity for bone regeneration.

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keywords: Polyhydroxyalkanoates, Bioactive glasses, Bone tissue engineering, Composite materials, Antimicrobial activity

73296319608

ALPHA TOCOPHEROL, ALPHA-TOCOPHERYL POSPHATE AND GN-2-NPM9, MOLECULES FOR THE MODIFICATION OF CHEMICALLY TREATED TI6AL4V ALLOY SURFACES FOR ANTIBACTERIAL AND ANTI-INFLAMMATORY PURPOSES.

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Introduction:
Implant infection, due to bacterial contamination, is a significant problem that represents one of the main causes of implant loss over time. In addition, the incidence of antibiotic resistance is steadily increasing, and alternative ways to fight or prevent infection have become the subject of biomedical research, and several surface modification and coating techniques have recently been developed for antibacterial applications.
In this work, three different molecules: alpha tocopherol, its water-soluble version alpha-tocopheryl phosphate, and a synthesized peptoid (GN-2-Npm9) were studied and chosen to create surfaces with antibacterial and anti-inflammatory properties.

Experimental Method:
Titanium Ti6Al4V alloy samples (ASTM B348, Gr5, Titanium Consulting and Trading, 10 mm diameter discs) were ground (up to 400 grit), then washed in acetone and deionized water. The discs were chemically treated to increase nanoscale roughness, to expose OH groups, and to make the surface more suitable for the grafting. The treated samples were irradiated with UV light to reduce carbon contamination. The modifications of titanium surfaces are explored as a coating or through functionalization and a procedure for proper characterization of this substrates was investigated. Physical and chemical characterization was performed through specific measurement techniques such as FTIR-ATR, Z-potential, reflectance spectroscopy, contact angle and release tests. Biological characterization was performed through cellular and antibacterial assays.

Results and discussion:
The modified surfaces are compared through FTIR-ATR and reflectance spectroscopy, z-Potential titration curves, contact angle measurements, release test, and tape test. The aim is to verify the effective presence of the molecules on the surfaces, the chemical stability over time, mechanical adhesion properties and hydrophobic behaviour for all three grafted molecules. The biological outcome is tested by the cellular and antibacterial tests. According to the results, both grafting and coating can be effectively performed and the biological response can be modulated from anti-adhesive to tissue integration by properly selecting the grafted biomolecules according to the final application (temporary or permanent implants). The surfaces show interesting antibacterial and anti-inflammatory properties.

Conclusion:
This work highlights a promising new application of these biomolecules as possible candidates for bone implant surfaces that reduce the risk of an excessive pro-inflammatory response, the risk of implant-associated infections, and allow for the disincentive of antibiotic use associated with the post-operative period.

keywords: Titanium, Implants, Antibacterial

62825450346

BIOACTIVE GLASSES WITH ANTIBACTERIAL PROPERTIES FOR BONE TISSUE REGENERATION

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Introduction
Since the discovery of bioactive glasses (BG) in the late 60s [1], the research in this area has significantly increased to obtain compositions with multiple functionalities not only from the materials aspect but also to provide a favorable biological response for tissue regeneration. BGs have the ability to react with the surrounding environment and bond to hard tissue. This property is characterized by the formation of a hydroxycarbonate apatite (HCA) layer on the material with a similar composition to the inorganic component of bones. Simultaneously, the dissolution of BG takes place leading to the release of biologically active ions, which stimulate the formation of new tissue at a cellular level. The incorporation of metallic ions in the BGs composition has been considered to obtain glasses able to stimulate the formation of bones, support biological processes such as angiogenesis and provide an antibacterial effect on the implantation site [2].

Methodology
Silicate-based BG glasses were produced with the melt-quench method. The well-known 45S5 BG composition was considered as reference material and new compositions were obtained incorporating zinc, boron and strontium. The bioactive behavior was assessed in simulated body fluid and characterized via FTIR, SEM and XRD. In vitro cell studies were carried out using pre-osteoblast cells MC3T3-E1. Moreover, the antibacterial effect of BGs was determined via indirect and direct experiments with Gram-positive and Gram-negative bacteria, S. aureus and E. coli, respectively. Turbidity measurements, counting colony-forming units and metabolic assays were used.

Results
The formation of an HCA layer on the material's surface was characterized by the detection of adsorption bands in FTIR spectra attributed to calcium phosphate and crystalline reflections of HCA. Which were also detected with XRD. The incorporation of the studied metallic ions delayed the formation of HCA compared to the non-doped 45S5 BG, which exhibited faster bioactivity after 1 day compared to 3 days for Sr-doped BGs and 7 days for B- and Zn-BGs. In terms of cell viability, the glasses containing boron and strontium outperformed the reference BG at all tested concentrations, whereas Zn-doped BG presented the lowest cell viability, particularly, at the highest BG concentration. The minimum inhibitory concentration of the dissolution products of BGs required to kill bacteria was lower for the Sr-doped BG and the reference 45S5 BG compared to the other glasses. However, all materials exhibited an antibacterial effect compared to the control sample (bacteria without BGs).

Conclusion
All studied BG compositions exhibited beneficial properties in terms of bioactivity and biological response. Although the bioactivity was slightly delayed, the in vitro biological assays showed a superior biological effect of Sr-doped BGs compared to control cells, control bacteria and to the other BGs. These results suggest a potential application of ion-doped BGs for bone regeneration.

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keywords: Bioactive glasses, antibacterial properties, bioactivity, bone regeneration

73296375448

DROP ON DEMAND: A NEW METHOD TO DEVELOP ANTIMICROBIAL COATINGS ON MEDICAL IMPLANTS

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Introduction: In the last years, partly given to the changes in the age structure of the population, there has been a skyrocketing increase of the number of orthopaedic surgeries [1]. With the rising number of implantations, the absolute number of complications is inevitably increasing at the same pace, causing not only distress for the patients but also a significant economic burden [2]. One of the major causes of implant failure is biofilm-associated infections, which represent a huge challenge given their high tolerance to antibiotic therapy. Drop on demand technology has proven to be a valuable tool to develop antimicrobial coatings to avoid bacterial attachment and biofilm development onto the implant surface. This technique enables the production of complex drug release profiles allowing a sustained release of antibiotics and biofilm inhibitors [3]. In this work, we developed PLGA antimicrobial coatings on titanium discs using drop on demand technology. N-(abiet-8,11,13-trien-18-oyl) cyclohexyl-L-alanine (DHA1) was used as a biofilm inhibitor.

Methodology: PLGA (PDLG 5004A, Corbion) and PEG (35 kDa, Sigma) were dissolved in organic solvents. DHA1 was added to the solution at different concentrations (10, 20 and 30% w/w). The viscosity of ink formulations was studied with a viscometer (DV-III Ultra, Brookfield). The printability of the different formulations was studied using a 3D Discovery (regenHU) inkjet microvalve-based 3D printer. The printing parameters were optimized, studying the pressure, opening valve time (OT), distance nozzle-sample, and nozzle diameter. The antimicrobial properties of this coating were studied by assessing its capacity preventing Staphylococcus aureus ATCC 25923 adhesion to the titanium surface.

Results: The viscosity and printability of different ink formulations were studied. A small range of viscosity was observed to obtain a good droplet formation and printability. A too high viscosity produced the clog of the nozzle, while a too low viscosity produced splashes and formation of satellites. The thickness of the coatings was in the range of 30-40 mm and 3 mg×cm-2 per layer. The presence of DHA1 in the coatings was confirmed by FTIR. The coatings loaded with different concentrations of DHA1 (10, 20 and 30% DHA1) reduced bacterial adherence up to 4-logs when compared with the titanium coated with PLGA-PEG with no load. Moreover, the released eluates from the coating with 30% DHA1 load managed to inhibit biofilm formation up to 24 h.

Conclusions: Drop on demand is a suitable technique for the development of antimicrobial coatings. The coating developed here proved high capacity preventing S. aureus biofilm formation. As far as we know, this is one of the first drop on demand coatings incorporating a biofilm inhibitor.

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Comment: Author 1 and 2 contributed equally to this work.

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keywords: drop on demand, antimicrobial, coatings, orthopaedics

41883661749

DEVELOPMENT OF MULTIFUNCTIONAL HYALURONIC ACID HYDROGELS WITH ANTIBACTERIAL, ANTI-INFLAMMATORY AND NUCLEIC ACID DELIVERY PROPERTIES

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Introduction
Implantation of biomedical devices is followed by immune response to the implant, as well as occasionally bacterial and yeast/fungi infections (1-3). In this context, new implant materials and coatings that deal with medical device-associated complications are required. Antibacterial and anti-inflammatory materials are also required for wound healing applications, especially in diabetic patients with chronic infected wounds. Such wounds are associated with high levels of pro-inflammatory cytokine secretion and iNOS production, contributing to non-healing phenotype.

Methodology
We have previously described thin films made of hyaluronic acid (HA) and polyarginine (PAR) that can be applied to all kind of medical devices. Such films constructed by layer-by-layer assembly demonstrated antimicrobial and anti-inflammatory properties (4-6). Recently, we presented antibacterial HA-based hydrogels cross-linked with 1,4-butanediol diglycidyl ether (BDDE). In HA hydrogels cross-linked with BDDE, carboxylic groups are preserved, and can be used for complexation with positively-charged antibacterial polymers such as PAR (7). Now, we used this system for multifunctional HA-based hydrogels.

Results
For the first time, we fabricated PAR/miRNA-loaded HA hydrogels with antibacterial and anti-inflammatory properties, which simultaneously act as miRNA delivery system (article submitted).
We demonstrate that PAR decreases inflammatory response of LPS-stimulated macrophages and accelerates fibroblast migration in macrophage/fibroblast co-culture system, suggesting a positive effect on wound healing. Furthermore, PAR allows to load miRNA into HA hydrogels, and then to deliver them into the cells. Potentially any miRNA can be used, making the system highly versatile. For instance, the hydrogels can be associated to different functional miRNAs, such as anti-inflammatory or angiogenic, to increase anti-inflammatory properties or to induce revascularization at the wound site.

Conclusion
To our knowledge, this study is the first describing miRNA-loaded hydrogels with antibacterial effect and anti-inflammatory features, making this system promising for infection treatment and foreign body response modulation. We believe that our system can become useful for the treatment of infected wounds such as diabetic ulcers, that are extremely difficult to heal and usually end up with amputations.

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keywords: biomaterials, hydrogels, antibacterial, anti-inflammatory, miRNA

20941802728

3D PRINTED SCAFFOLDS WITH NON-ANTIBIOTIC ANTIMICROBIAL-DOPED HYDROXYAPATITE FOR INHIBITING S. AUREUS GROWTH IN VITRO AND SUPPORTING BONE REGENERATION IN VIVO

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Introduction
Bone infections (osteomyelitis) are difficult to treat due to the formation of biofilms, antibiotic resistance, and limited penetration of systemic antibiotics to infection site(1). Highly-porous collagen-hydroxyapatite (C-HA) scaffolds have proven capacity to regenerate critical-sized bone defects in vivo and human clinical trials(2). However, they still lack appropriate mechanical properties to support larger defects and weight-bearing applications(2). The objective of this study is to develop a reinforced non-antibiotic antimicrobial-doped hydroxyapatite scaffold to overcome issues surrounding antibiotic resistance, while providing a suitable environment for bone regeneration. The aims are to (i) optimise the delivery of metal-based antimicrobials from the scaffold without impeding pro-osteogenic properties in vitro, (ii) reinforce the metal-based antimicrobial scaffold using 3D printing to enhance the mechanical properties, and (iii) evaluate the reinforced scaffold system in a weight bearing rat femoral defect in vivo.

Methods
A range of metal-based antimicrobial doped hydroxyapatite (MBA-HA) doses (formulations not disclosed due to IP restrictions) were incorporated into type-1 collagen matrix to fabricate collagen-MBA-HA (C-MBA-HA) scaffolds. The C-MBA-HA scaffolds were evaluated for cell viability and osteogenesis using rat mesenchymal stromal cells in vitro up to 28 days. The scaffold's antibacterial properties against S. aureus were assessed in vitro. A 3D-printed polymer framework was combined with the collagen matrix for reinforcement. The compressive modulus, porosity, and microarchitecture were assessed. The reinforced scaffolds were further evaluated in vitro to ensure there were no negative affect of reinforcement on the osteogenic

and antimicrobial capacity. Ongoing evaluation of the bone healing potential of C-MBA-HA is being assessed in a 5mm long critical-sized rat femoral defect and will be compared against C-HA and empty defects at 2,4, and 8 weeks using micro-computerisation tomography (μCT) and histology at 8 weeks.

Results
Biomimetic C-HA scaffolds were successfully functionalized with MBA-HA to achieve antimicrobial properties while continuing to support osteogenesis. Specifically, the C-MBA-HA scaffolds resulted in equivalent calcium deposition to the C-HA scaffolds at 28 days, which was further validated with the homogenous distribution of alizarin red staining, i.e., revealing cell-mediated mineralization throughout the scaffolds. The C-MBA-HA scaffolds achieved a 50% reduction of S. aureus, as well as the development of inhibition zones on S. aureus agar plates after 24 hours. The 3D-printed polymer framework was successfully integrated into the C-MBA-HA scaffold to significantly enhance its mechanical properties to better mimic cancellous bone, while maintaining high porosity and an microarchitectural structure favourable for cellular infiltration and bone formation. In vitro cellular and microbial assessment of the reinforced C-MBA-HA scaffolds demonstrated no reduction in beneficial properties when compared to the non-reinforced scaffold.

Conclusion
The successful development of this non-antibiotic antimicrobial and osteoconductive scaffold with enhanced mechanical properties for treatment of weight bearing large defects has the potential to be a one-step local treatment for osteomyelitis.

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keywords: Bone infection, biomaterials, non-antibiotic antimicrobials, 3D printing

S09
Biobanking - indispensable support for the development of regenerative medicine
Room: S4 B (28 Jun 2022, 13:30 - 15:00)

Conveners:
Anna Chróścicka; Maria Chatzinikolaidou

31451702248

CAN BIOFABRICATION TECHNOLOGIES HELP TO FACILITATE BIOBANKING OF TISSUE ENGINEERED PRODUCTS?

Lorenzo Moroni (Maastricht University - MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration department, Maastricht, Netherlands)

Organs are complex systems, comprised of different tissues, proteins, and cells, which communicate to orchestrate a myriad of functions in our bodies. Technologies are needed to replicate these structures towards the development of new therapies for tissue and organ repair, as well as for in vitro 3D models to better understand the morphogenetic biological processes that drive organogenesis. To construct tissues and organs, biofabrication strategies are being developed to impart spatiotemporal control over cell-cell and cell-extracellular matrix communication, often through control over cell and material deposition and placement. These technologies could also play a role in protecting seeded or encapsulated cells in case of off-the-shelf products are desired for biobanking purposes.
Here, we present some of our most recent advancements in biofabrication that enabled the control of cell activity, moving towards enhanced tissue regeneration as well as the possibility to create more complex 3D in vitro models to study biological processes, with a particular attention to biobanking requirements to preserve tissue construct functionality before and after storage.

keywords: Biofabrication, Stem Cells, Biomaterials, Regenerative Medicine

94355107448

TISSUE ENGINEERING AND BIOBANKING - A POSSIBLE FORCE-JOINING ALLIANCE IN APPLIED SCIENCES

Malgorzata Lewandowska-Szumiel (Medical University of Warsaw, Warsaw, Poland)

Almost 160 clinical trials of tissue engineered products (TEPs) currently registered in ClinicalTrials.gov databases as well as the continuously growing record for TEPs under clinical investigations starting from the year 2006, can be taken as one of the many measures of success of tissue engineering as an applied science discipline. On the other hand, the number of TEPs on the medical market remains at a level that can be considered below expectations. Obviously, the road of the medicinal products to the market is costly and time consuming. Extremely complicated, unstable and demanding regulatory issues concerning advanced therapy medicinal products make it even more difficult. Nevertheless, it is hard to disagree that - so far - TEP-based therapies have failed to bring medical breakthroughs. Among the substantive reasons, one of the most important seems to be the multiplicity of variables at all stages of design and validation of TEPs. On one hand it opens more possibilities, but on the other one, it often limits repeatability of results. The reproducibility of the active substance of TEPs, i.e. the cells, is already extremely difficult to achieve. Even when the requirements of pharmaceutical law are met, the donor-dependent biological diversity of the cells makes it difficult to obtain reproducible results. Advanced preclinical studies of TEPs in vitro, repeated on material from many donors, may bring significant progress. At this point, tissue engineering come close to biobanking, which meets this need. By the most general terms, biobanking means collecting all types of biological material, such as blood, tissue, cells, RNA or DNA, preferably in connection with the data related to the samples. For health research, biobanking material of human origin is now considered an extremely important tool. In particular, acquisition, preparation and storage of well-characterized biological material from multiple donors - under the control of quality standards - is the response of the scientific community to reports stigmatizing the lack of repeatability of preclinical studies. Strong collaboration between tissue engineers – model beneficiaries and at the same time potential suppliers of biological material for biobanks and biobankers - who provide biological material of confirmed quality necessary for research, opens new possibilities for the development of well-defined TEPs of repeatable properties.
Some examples illustrating the problems mentioned here, based on our own experience from clinical trial on TEPs carried out with the participation of 100 volunteers will be discussed. On the other hand, the activities of BBMRI-ERIC - a European research infrastructure for biobanking will also be presented. The combination of both of these issues will be shown in the context of a possible common added value of significant importance for medical research.

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Comment: Invited speech for the Symposium: "Biobanking - indispensable support for the development of regenerative medicine"

keywords: tissue engineered products, clinical trials, advanced therapy medicinal products, biobanking

62825471109

ARE THERE ANY DIFFERENCES BETWEEN BIOBANKING AND BANKING OF TISSUES AND CELLS FOR CLINICAL USE?

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Biobanking refers to the process by which samples of human body fluids, tissues or cells are collected to provide samples for different research purposes. Initially, the term biobank appeared in the scientific literature to describe human population-based biobanks.
Biobank is nowadays recognised as an organized collection of samples and associated data describing donor, stored for future research in biomedicine.
A tissue and cel bank is an establishment that and procure human tissues and cells from deceased and living donors which are than processed, stored and distributed for allo- and autograft transplantations.
A problem is raised when human tissues and cells are used in clinical studies. How the procedurę of Transplantation should be clasified, what are legal requirements to be followed by bank? Does bank of tissues and cells became a biobank. What are the conditions, if biobank distribute tissues or cells for clinical use? Is it possible that biobank distribute tissues or cells as starting materils for manufacturing of advanced therapy medicinal products (ATMP) including tissue engeenired productcs or for gene therapy?

keywords: biobanking, banking of tissues and cells, starting materials for ATMP

52354574349

BONE-FORMING CAPACITY AND IMMUNOGENICITY OF ENGINEERED AND DECELLULARIZED HUMAN CARTILAGE GRAFTS

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Introduction: The surge in clinical need for bone tissue restoration together with limitations of existing treatments calls for the development of alternative strategies. Tissue engineering has been proposed towards the formation of bone graft substitutes capable of driving repair. Previously, we demonstrated the possibility of generating engineered cartilage grafts using a human mesenchymal stromal cell (hMSC). Here, we aim to develop an efficient decellularization protocol and assess the osteoinductivity, immunogenicity and regenerative potential of decellularized cartilage in both immunodeficient (ID) and immunocompetent (IC) settings.

Method: Our graft consists of in vitro engineered cartilage tissue produced by human mesenchymal stromal cell (hMSCs) lines. After cartilage formation, the tissue was subsequently decellularized using a combination of hypertonic and hypotonic baths, Sodium dodecyl sulfate (SDS), and DNase to effectively remove cells, thus resulting in a cell-free graft aiming at instructing bone formation by endochondral ossification. The decellularized cartilage is implanted subcutaneously in the back of IC and ID animals for a maximum of 12 weeks. The early recruitment of immune cells (dendritic cells, monocytes, macrophages, natural killer, T and B cells) was assessed quantitively by flow cytometry at 3-, 7- and 10-days post-implantation (Immune prints).

Results: We demonstrated the reproducible engineering of decellularized human cartilage, as cell-free grafts capable of bone formation by exploiting a dedicated human Mesenchymal Stromal Cell line (MSOD-B). Following subcutaneous in vivo implantation, a complete remodeling into bone was achieved in immunodeficient mice (ID) through recapitulation of the endochondral ossification pathway. In contrast, only minor calcification was observed upon implantation into immunocompetent mice (IC). Initial immune responses during successful bone formation in ID seemed to correlate with an early M2 macrophages polarization and recruitment. Moreover, we showed that despite the absence of cellular material, human-derived grafts were able to induce a pro-inflammatory response in IC, detrimental to effective bone formation.

Conclusion and discussion: Using our decellularization method, we efficiently removed significant amount of cells without affecting the overall structure and composition of cartilage graft. Ectopic evaluation of decellularized tissues displayed excellent osteoinductive properties, correlating with early M2 polarization in ID mice but not in IC mice. This indicates ECM interspecies variations could still result in the immune rejection of cell-free tissues. Compiling immune prints may offer understanding the immunogenicity of engineered grafts, and help designing biomaterials with tailored immune profile for effective repair.

keywords: Bone remodeling, Immunology, in vitro cartilage

20941857789

LIPID-POLYMER NANOCARRIERS FOR CARTILAGE REGENERATION

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Introduction: Musculoskeletal ailments caused by cartilage damage are common, and thanks to more modern diagnostic methods, they are more often recognized. Moreover, cartilage diseases progress with age and result from injuries, becoming a dominant problem in orthopedic surgery. The conducted research aims to produce conjugates for cartilage regeneration based on chondrogenic differentiation of mesenchymal stem cells isolated from Wharton's jelly (hUC-MSC). Composed conjugates are based on lipid carriers of kartogenin and glycosaminoglycans derivatives stabilize the whole system.

Methodology: Kartogenin (KGN) – an active substance – was encapsulated in the liposomes. The lipid carriers were covered with hydrophobically modified chondroitin sulfate (CS) or hyaluronic acid (HA). The physicochemical analysis of the obtained systems was carried out by dynamic light scattering, zeta potential, and fluorescence measurements. The thermotropic behavior of lipid membranes was studied using a Nano DSC calorimeter (TA Instruments). In addition, the interactions of polymers with liposomes (loaded or unloaded by KGN) were analyzed by microscale thermophoresis (Monolith, Nanotemper). hUC-MSC morphology imaging after incubation with the prepared formulations and metabolic toxicity test were performed. Additionally, these systems have been tested to differentiate stem cells into chondrocytes using real-time PCR (rt-PCR).

Results: CS and HA substitution by alkyl domains were confirmed by XPS spectra. KGN was successfully incorporated into the lipid bilayer. Composed formulations were stabilized by covering their surfaces with CS or HA derivatives. The changes in thermograms for an aqueous dispersion of DPPC confirmed the incorporation of polymers' hydrophobic domains into the lipid bilayer. Moreover, an increase in the liposome size and decrease in the zeta-potential values confirmed the presence of polymers on liposomes surfaces. Despite both systems: modified CS and liposomes with KGN have high negative charge, they interact because of the hydrophobic effect. According to cytotoxicity results (MTT assay), all polymers used in lipid formulations were significantly non-toxic, than pure polymers. This is explained by the fact that hydrophobic anchors are hidden in the lipid bilayer and their exposure to the cell surface is minimized. The selected genes expression was analyzed by real-time PCR. All systems have induced ACAN and SOX9 gene expression compared to untreated cells.

Conclusion: Composed hybrid lipid-polymer formulations are stable vesicles of KGN. Moreover, they can be embedded into hydrogels and provide control released of the cargo. The resulting systems are promising conjugates for the regeneration of cartilage tissue.

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keywords: kartogenin, liposome, glycosaminoglycans, stem cells, cartilage

52354563999

THE EFFECT OF AUXETIC METAMATERIAL SCAFFOLDS IN OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Introduction
Severe bone injuries can result in incapacities and thus affect a person's quality of life. Mesenchymal stem cells (MSCs) can be an alternative for bone healing by growing them on scaffolds that provide mechanical signals for differentiation. Such scaffolds can give the appropriate ques to the cells in order to induce their differentiation into mature osteoblasts and later on to be transplanted into the body. Until now lots of attention has been given to create appropriate nano and micro patterns that can work as signal inducers limiting the research in only 2.5 dimensions. On the other hand, our work introduces true, well- defined 3D environments to the research of MSCs differentiation.

Methodology
In our approach, we fabricated hierarchical auxetic mechanical metamaterials and ultra-light ultra-stiff scaffolds via two photon polymerization and used them as scaffolds to investigate the differentiation of MSCs into osteoblasts. Those scaffolds consist of unit cells comparable to the diameter of MSCs which is approximately 50 to 100μm, so only a couple of cells can fit inside thus ensuring the optimal mechanical environment for each cell. In the case of auxetic scaffolds, the unit cells are able to bend without breaking such that the cells can adapt their environment to their needs, whereas the kelvin foam is stiff non elastic scaffold that shows no deformation in response to the forces exceeded by the cells. We investigated the localization of YAP protein, a key protein transcription factor that acts as a mechanotransduction mediator and compared it to common osteogenic markers in both protein and gene levels by using confocal microscopy and qPCR .

Results
Interestingly, YAP protein is translocated to the nucleus even after 21 days of culture and RUNX2 gene shows a 10-fold increase in auxetic scaffold in comparison with the control only after 7 days. Long term cultures up to 28 days shows high mineralization of the extracellular matrix after Alizarin red staining. Moreover, SEM pictures revealed different cell morphology in those different scaffolds because of the different geometries used. Auxetics pushes the cells into more elongated phenotypes whereas kelvin foam in more broad cells bodies.

Conclusion
Auxetic scaffolds are ideal for osteogenic differentiation as they can maintain and promote the osteogenesis efficiently even after 28 days of culture. Our work paves the way for the use of more complicated metamaterials into the tissue engineering field.

Acknowledgement
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keywords: auxetic, metamaterials, osteogenesis, differentiation, multi photon lithography

20941850409

CRYOPRESERVED ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION FOR THE GROWTH FACTOR-FREE VASCULARIZATION OF BLUE SHARK COLLAGEN SPONGES

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Introduction:
Vascularization is a critical aspect of every tissue engineering (TE) approach, especially in 3D constructs. The formation of a network of capillaries is necessary to ensure adequate delivery of nutrients and oxygen to cells within the constructs, as well as fast anastomosis with the host's vasculature after implantation. Pre-vascularization of these constructs before implantation can be a solution. However, cell sourcing is a limiting issue. Adipose tissue is regarded as a privileged source of mesenchymal progenitor cells due to its easy accessibility and abundancy. This tissue hosts adipocytes, as well as a stromal vascular fraction (SVF) comprising several other cell types including fibroblasts, endothelial progenitors, endothelial cells and hematopoietic cells. Due to this composition, the SVF of adipose tissue is highly angiogenic and has been proposed for the growth factor-free vascularization of TE constructs[1]. To produce such constructs, collagen from mammalian sources is widely used. However, regulatory issues associated with the risk of disease transmission have boosted the search for new collagen sources such as from marine organisms. Collagen from otherwise wasted blue shark skin was herein used to produce sponges that were then seeded with cryopreserved SVF for growth factor-free vascularization.

Methodology:
A blue shark skin collagen hydrogel was created by acid solubilization of blue shark skin collagen, followed by cryogelation with crosslinking reaction carried out at low temperatures using 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride. Finally, cryogels were freeze dried to form the collagen sponge. SVF was isolated from human adipose tissue subcutaneous tissue and cryopreserved in 10% DMSO in FBS with a controlled freeze rate of 1ºC/min, for at least 7 days. SVF was then seeded on the collagen sponges and cultured for 7 days to create a pre-vascularized construct. Sponges' pre-vascularization was assessed in vitro by immunohistochemistry and secretome profiling and their functionality was tested in ovo using a chick chorioallantoic membrane (CAM) assay.

Results:
After 7 days of in vitro culture, CD31 expression pattern demonstrated the formation of a vessel like network. The secretome profile of angiogenic-related factors changed with culture time. From 5 to 7 days of culture, there was an increase in the secretion of both pro angiogenic

proteins (VEGF, MMP-9, IL-8) and angiogenesis inhibitors (TIMP-1, SERPIN E1, Thrombospondin-1). Upon in ovo implantation, vessel number quantification demonstrated an increase in vessel recruitment in pre-vascularized sponges when comparing with sponges without SVF cells. CD31 expression pattern demonstrated the integration of the pre-vascular network within the CAM, while in situ hybridization confirmed the presence of the seeded human cells.

Conclusions:
These results demonstrate the potential of cryopreserved SVF to assist in the vascularization of TE constructs in an extrinsic growth factor-free manner, allowing a simplified and cost-efficient methodology to ensure construct integration after implantation.

1. Costa, M et al, Acta Biomater. 2017 Jun 1;55:131–43.

Acknowledgements:
EU Horizon2020 ERC grant CapBed (805411); FCT fellowships PD/BD/135252/2017, IF/00347/2015; INTERREG España-Portugal 2014-2020 project 0474_BLUEBIOLAB_1_E; Atlantic Area Programme project BLUEHUMAN (EAPA_151/2016) and NORTE2020/PT2020 project ATLANTIDA (Norte-01-0145-FEDER-000040). Dr. Cármen G. Sotelo (IIM-CSI, Vigo, Spain), for the kind offer of blue shark skin collagen.

keywords: Shark collagen sponges; Stromal Valcular Fraction; Vascularization

S10-1
Biofabricated Tissues and Organs for Clinical Impact
Room: S1
(29 Jun 2022, 11:00 - 12:30)

Conveners:
Andrew Daly; Laura De Laporte

62903404977

BIOFABRICATED ARTICULAR AND CARDIAC TISSUES FOR CLINICAL IMPACT

Jos Malda (UMC Utrecht, Utrecht, Netherlands)

Three-dimensional (3D) printing is already routinely used in the clinic, e.g. for pre-operative models or intra-operative guides. However, this does not involve the generation of living 3D structures, i.e., biofabrication of tissues and organs. This automated approach holds potential to advance the field of regenerative medicine as outer shapes can be personalised and organised constructs can be produced when printing with multiple bio-inks. Recent developments have now resulted in the availability of a plethora of bioinks, new printing approaches, and the technological advancement of established techniques. Nevertheless, mimicking the functional properties of the tissues and clinical translation of the technology are two important remaining challenges. In order to achieve this, we urge that the field now shifts its focus from materials and technologies towards the biological development of the resulting constructs. Moreover, there is an urgent need for more specialized production facilities to move this technology towards the patient.

keywords: bioprinting, biofabrication, cartilage, osteochondral

52354545999

PHYSIOMIMETIC CULTURE OF MESENCHYMAL STROMAL CELLS AFFECTS MACROPHAGE ACTIVITY IN A PARACRINE MANNER

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Introduction:
Mesenchymal stromal cells (MSC)-based therapies for inflammatory diseases rely mainly on the paracrine ability to modulate different cell populations involved in the advance of the disease, such as macrophages. These immune cells possess a broad spectrum of inflammatory responses. In addition, previous data have shown that the MSC secretome influences macrophage phenotype and functional capacities. Furthermore, culturing MSC with physiomimetic cues from the extracellular matrix (ECM) have shown to improve their repairing actions upon transplantation. Physiomimetic culture of cells relies mainly on ECM-derived biomaterials, such as, decellularised scaffolds and lung ECM hydrogels which provide a similar biomechanical milleau to the organ. Despite the recent advances in MSC-based therapies, there is scarce information regarding the changes on the secretome content attributed to these culture platforms, and especially, how the secretome profile could influence macrophage activity in favour of therapy. In this setting, the aim of this study was to assess the macrophage activity exerted by the secretome isolated from physiomimetically cultured lung-resident mesenchymal stromal cells (LMSC).

Methodology:
LMSC from human donors were cultured on in-house developed devices that enable lung-mimetic strain. Medium from LMSC cultured in either lung ECM scaffolds and in lung ECM hydrogels whilst subjected to cyclic stretch, and on tissue culture plates (TCP) was analysed for typical cytokines, chemokines and growth factors. RNA was analysed for the gene expression of relevant mechano regulators CTGF and CYR61. Human monocytes were differentiated to macrophages by adding PMA and assessed their phagocytic capacity of bioparticles in the presence of LMSC secretome. Macrophages were also polarized to M1 and M2 phenotypes by adding LPS or IL-4 plus IL-10, respectively. M0 (quiescent), M1 and M2 macrophages were exposed to the medium of LMSC from the different culture conditions and analysed for surface markers by flow cytometry.

Results:
CYR61 gene expression showed decreases when cultured on the aforementioned lung-mimetic environments compared to TCP. Furthermore, CTGF and CYR61 displayed a marked reduction when cultured in lung ECM hydrogels. The secretome content was plotted in UMAPs where the scaffold clusters mostly to itself while there is a large overlap between the hydrogel and the TCP samples. Additionally, stretch elicited different changes on HGF, MCP-1, IL-6 and TNF-α according to the environment where LMSC had been cultured. Similarly, phagocytosis showed a differential increase on TCP due to the stretch which was not observed in the physiomimetic culture.

Conclusion:
Mechanical features of the lung ECM orchestrate key outcomes on LMSC, hence providing new insights into preconditioning of MSC for therapy.

keywords: Lung Bioengineering, Physiomimetic Culture, Therapy

31412740986

ENGINEERED AND DECELLULARIZED HUMAN CARTILAGE GRAFTS INSTRUCT FULL REGENERATION OF CRITICAL-SIZED FEMORAL DEFECTS

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Introduction: Recent bone tissue engineering strategies propose recapitulating the endochondral ossification process for an effective repair. To this end, primary human mesenchymal stromal cells (hMSCs) can be primed in vitro towards hypertrophic cartilage (HyC) formation. While holding promises, limits arise from the performance variability associated with the use of primary cells. Recently, we developed a customized hMSC line, the MSOD-B, over-expressing BMP-2 and capable of reproducible cartilage formation in vitro. Following devitalization, the tissue exhibited remarkable osteoinductive properties. Here, we aim to move one step closer to clinical translation by investigating the possibility of decellularizing our cartilage graft and assessing its in vivo regenerative capacity in a rat critical-sized femoral defect.

Method: Our graft consists of in vitro engineered cartilage tissue produced by the MSOD-B. Subsequently, the tissue is decellularized by a combination of hypertonic/hypotonic, detergent (SDS), and DNase washes. The osteoinductivity of decellularized constructs was assessed through subcutaneous implantation in immunodeficient (ID) mice. To evaluate their repair capacity, , decellularized constructs were implanted in a critical-sized femoral defect (5-mm) in Sprague Dawley rats. Repair was assessed 6- and 12-weeks post-implantation through histological, micro-computed tomography (μCT), and mechanical analyses.

Results: We demonstrated the reproducible engineering of decellularized cartilage by exploiting a mesenchymal line. Decellularization resulted in a drastic reduction of DNA (<100ng/construct) with a minimal impact on tissue structure and composition (collagen, GAGS, and embedded growth factors). Remarkably, the capacity to instruct bone formation by endochondral ossification of our decellularized cartilage was not affected. This was validated both at ectopic site and in a critical-sized femoral defect in an immunocompetent rat model, with full-bridge after six weeks and complete bone repair observed 12 weeks post-implantation.

Conclusion and discussion: Our study illustrates the capacity of exploiting customized human lines to produce osteoinductive decellularized extracellular matrices (dECM). The strategy offers both standardization of performance and unlimited tissue availability, opening new avenues for the manufacturing of dECM for bone repair.

keywords: Bone, Tissue Engineering, Decellularization

94238145786

A WOVEN VASCULAR GRAFT PRODUCED FROM YARN OF HUMAN AMNIOTIC MEMBRANE

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Since synthetic vascular prosthesis perform poorly in small diameter revascularization, biological vascular substitutes are being developed as an alternative. Although their in vivo results are promising, their productions involve tissue engineering methods that are long, complex and expensive. To overcome these limitations, we propose an innovative approach that combines the human amniotic membrane (HAM), which is a widely available and cost-effective biological raw material, with a rapid and robust textile-inspired assembly strategy. [1] Fetal membranes were collected after cesarean deliveries at term. Once isolated by dissection, HAM sheets were cut in ribbons that could be further processed, by twisting, into threads.

Characterization of HAM yarns (both ribbons and threads) showed that their physical and mechanical properties could easily be tuned. Since our clinical strategy will be to provide an off-the-shelf, allogeneic implant, we studied the effects of decellularization and / or gamma sterilization on the histological, mechanical, and biological properties of HAM ribbons. Decellularization had little effect of HAM yarn mechanical properties other than a small increase in strain at failure. However, gamma sterilization of the dried and decellularized HAM caused a decrease in rehydrated yarn diameter, an increase in ultimate tensile strength and a decrease in strain at failure. Gamma irradiation of hydrated (and decellularized) HAM largely avoided these mechanical changes and the process did not interfere with the ability of the matrix to support endothelium formation in vitro.

Finally, HAM-based, woven, tissue-engineered vascular grafts (TEVGs) showed clinically relevant mechanical properties with a burst pressure of over 8000 mmHg (at a diameter of 4.4 mm), suture retention strength of over 5 N, and a transmural permeability of 1 ml·min-1·cm-2 Thus, this study demonstrates that human, completely biological, allogeneic, small diameter TEVGs can be produced from HAM, thereby avoiding costly manufacturing strategies based on cell culture and complex bioreactors.

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as a truly “bio” material for tissue engineering applications. Acta Biomater, (105), 111-120, doi: 10.1016/j.actbio.2020.01.037 (2020).

keywords: Human amnionic membrane, vascular graft, woven, human textile

41883621846

PHILOSOPHY OF SCIENCE, A TOOL TO FACE ENGINEERED LIVER CHALLENGES

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Introduction
To overcome organ shortage, designers develop engineered livers: devices/methods aiming to temporally assist or permanently replace it. As a complex organ with more than 500 functions, the design of engineered livers is one of the greatest challenges of the field. Since the mid-20th century, multiple pathways have been taken using diverse materials such as charcoal or cells. We argue that establishing a design strategy to engineer a liver is not entirely a technical issue[1]. Our research aims to highlight all the factors and thus should give new design directions to engineered organ designers.

Methodology
We undertook a philosophical analysis guided by the literature and starting from the field. We conducted participant observation in the lab experiments and 24 semi-structured interviews with 19 international actors of this engineered organ field. A thematic method[2] was employed to analyze the data by distributing the interview's material into groups such as design strategy or regulation.

Results
We identified two major designers' sources of inspiration. First, their vision of the liver, which could be defined by its functions and/or structure. Secondly, the technologies/methods that the designer mastered. In addition, several constraints such as budget or regulation had an impact. Based on those influences, designers developed design strategies leading to different engineered livers such as artificial, bioartificial, or hybrid. Each designer had the same mantra "keep it simple" meaning "find the shortest path to the patient". Hence, if liver detoxification functions and artificial kidney were designers' inspirations, the simplest strategy was to avoid living components that complicated and lengthened the research and the industrialization process. Such a path led to an artificial device, which couldn't improve patients' survival. Also inspired by cellular culture, some designers changed their strategy: turn the dialysis filter into a bioreactor by adding cells, betting that the hepatocytes will perform their functions as in vivo. Such a path led to a bioartificial device, which was so far not more successful. Organ decellularization/recellularization mobilized the same betting strategy but designers' vision of the liver was more structural, speculating that if the structure was right, the function should follow. Recellularization remained however complex for large scale organs.

Conclusion
Highlighting the influences behind the technical choices and the resulting strategies can open up designers to innovative engineered livers. Grasping a device's nature is the first step towards understanding its ethical impacts, thus helping the legislators adapt the regulatory categories with requirements suited. Even if this research focuses on the liver, its conclusions could be valid for other organs.

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keywords: philosophy of science, engineered organs, design, liver

73296369939

TOWARDS FABRICATION OF A TRIPLE CULTURE LIVER SINUSOID MODEL UTILIZING 3D CORE-SHELL BIOPRINTING

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Introduction
In vitro liver models allow investigation of the cell behavior in disease conditions or in response to changes in the microenvironment and are therefore valuable tools for basic research, drug screenings or toxicological analyses. Mimicking the tissue-level complexity of liver to achieve functional constructs is a major challenge, however, 3D bioprinting technologies open novel options to recreate the liver microarchitecture. Previously, we have demonstrated the high potential of coaxial extrusion-based 3D bioprinting to establish patterned co-cultures of hepatocytes and fibroblasts in core-shell fashion [1]. The aim of the present study was to develop a liver sinusoid-like model consisting of a vascularized core compartment which is surrounded by a hepatocyte-laden shell compartment. A suitable core bioink was developed, bioprinting of the triple culture model was established and cell-cell interactions were observed.

Methodology
The shell bioink consisted of 3 wt% alginate and 9 wt% methylcellulose dissolved in fresh frozen plasma (plasma-algMC) as described [2]; HepG2 were added immediately prior to printing. The core bioink was prepared by mixing collagen, fibrinogen and gelatin to achieve a CFG blend with final concentrations of 1.33 mg/ml C, 5 mg/ml F and 4 wt% G. Human endothelial cells (HUVEC) and fibroblasts (NHDF) were mixed into the ink immediately prior to printing. Core-shell bioprinting was conducted using a Bioscaffolder 3.1 (GeSiM) equipped with a coaxial needle as described [1]; crosslinking of the bioprinted constructs was done in 100 mM CaCl2 supplemented with 0.3 U/ml thrombin solution. Cell viability was examined by live/dead staining, cell proliferation was investigated by cell number quantification and EdU staining. Immunostaining was conducted to visualize endothelial tube formation in the core and to prove hepatocytes biomarker expression in the shell. Albumin secretion was analysed by ELISA.

Results
Core-shell constructs consisting of the CFG bioink as core and the plasma-algMC bioink as shell were nicely printable and, after dual crosslinking, stable during further cultivation over at least 21 days. Plasma enhanced viability and supported proliferation, cluster formation and biomarker expression of HepG2 in the alginate-based shell compartment. Using NHDF as supportive cells, HUVEC formed a pre-vascular network in the CFG core. This network formation was also visible in the presence of HepG2 in the shell compartment, however, a competition of the HepG2 for the matrix-forming fibroblasts in the adjacent compartment was observed if a high number of HepG2 was used. The presence of HUVEC in the core compartment resulted in an increased albumin secretion of HepG2 in the shell compartment.

Conclusion
Based on core-shell bioprinting, a patterned triple culture model has been established which

can be further developed towards a more physiological liver sinusoid model.

References
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Acknowledgements
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keywords: liver, bioprinting, hepatocytes, angiogenesis, endothelial cells,

20941830879

LIVER MATRIX AND PERFUSION BIOREACTOR CULTURE PROMOTE AMNION EPITHELIAL CELL DIFFERENTIATION INTO FUNCTIONAL HEPATOCYTES

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Introduction: Mortality caused by liver disease and its complications is on the rise, representing a significant global health issue. Transplantation is the only efficient treatment for end-stage liver disease but is limited by the shortage of organ donors. Bioengineering represents a promising option, with researchers aiming at developing suitable organ replacements for transplantation. Tissue engineered organs rely (i) on a proper source of cells, able to support organ's functionality long term, and (ii) on bioreactors, for the culture of whole-organ constructs. Amnion epithelial cells (AECs) can be isolated from full-term membrane with no ethical concerns. AECs can mature into hepatocyte-like cells (HLC), representing a promising source of hepatocytes for liver regenerative medicine and toxicological evaluations. Extracellular matrix (ECM) proteins promote cell maturation and long-term function; organ-specific ECMs can be obtained using decellularization, which allows eliminating cells from a tissue while maintaining ECM composition and 3D-architecture.
Here, we induced maturation of human AEC into functional HLC by culturing the cells into a 3D decellularised liver construct in a custom-made bioreactor, and evaluated differentiation and functionality of the HLC obtained.

Methodology: 40-50 million human AEC (isolated from full-term amnion membrane and characterized via FACS and qPCR) were seeded into decellularised rat liver scaffolds obtained via established detergent-enzymatic treatment. Constructs were cultured in custom-made bioreactors for up to 40 days (10 in expansion and 30 in hepato-specific culture conditions), with static cultured scaffolds used as control. Metabolites (e.g. lactate and glucose) and hepatic activities were monitored at different time points via NMR, ELISA and EROD assays, and compared to primary human hepatocytes. Transcriptome and proteomic analysis at intermediate and final time points were used to confirm the functional analysis.

Results: Freshly isolated AECs were positive for epithelial markers but negative for mature hepatocytes markers. AEC seeded in ECM-scaffolds adhered and proliferated to some extent when exposed to proliferative conditions. After 2 weeks in hepatic maturation media, AEC expressed immature hepatocyte marker alpha-feto protein, while 2 additional weeks generated CK18+-HLC characterized by secretive (Albumin) and functional (CYP3A4) protein expression. Albumin and urea concentration in the culture media increased in bioreactor-cultured constructs in respect to static culture, and NMR showed a shift in metabolite production over the course of the maturation. Finally, phase-1 hepatic metabolism was quantified via EROD assay at different time points.

Conclusion: Here we show that liver ECM-scaffolds efficiently supported the maturation of AEC into functional HLC in a 3D liver model. Remarkably, both cell distribution and hepato-specific activities and functionality were enhanced when human AEC were cultured in bioreactor than in

static ECM scaffold. The bioreactor technology may provide an advantage for cell differentiation thanks to a more even distribution of oxygen and nutrients in comparison to static conditions. The technology here presented can serve as a paradigm for hepatic maturation in a 3D model of the liver composed by natural ECM and can help to investigate the role of ECM-specific protein in cell maturation and functionality.

keywords: amnion epithelial cells, decellularisation, bioreactor, liver regeneration

31412722057

A MODULAR BIOREACTOR FOR DYNAMIC CULTURING OF HUMAN MULTILAYER TISSUES STRUCTURES

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Introduction: Dynamic culturing systems can overcome challenges of in-vitro fabrication and maintenance of complex 3D tissues, however, the unique physiological conditions to which each tissue is subjected has been hampering noteworthy developments for many engineered tissues. Here we report the in-house development (design, manufacturing, and validation) of a glass slide size microbioreactor for the preparation, maintenance, and/or conditioning of human multilayer tissues or multi-tissue structures, evidencing applications in skin tissue-engineered analogs and ex-vivo human skin.

Methodology: The autoclavable bioreactor comprises a sandwich modular structure of hard undeformable layers of 3D printed medical grade polycarbonate intercalated with soft deformable layers of silicone. When compressed, the soft layers expand laterally against the sample sealing the layers between fluid streams avoiding their intermixing [1]. The bioreactor is modular, each module being an independent fluid circuit for one tissue, and up to three modules can be assembled to enable multi-tissue structures cultured in dynamic conditions. Each fluid stream is regulated by an independent piezoelectric pressure controller coupled with a flow sensor that allow measuring the dispensed media.

Results: The bioreactor was capable of holding skin tissue samples of 8mm in diameter and of providing, without mixing, different culture media corresponding to the three layers of the tissue, the outmost epidermis, the underneath dermis, and the innermost adipose tissue. By changing the thickness of the soft layers, it could easily be adapted to accommodate samples from different anatomical regions and with varied thicknesses. The bioreactor allowed nourishing each cell type/tissue layer with a specific cell culture medium, increasing the maintenance time of the native structure in the ex-vivo skin. Furthermore, the bioreactor permits establishing an air-liquid interface for epidermis turnover in the skin explant, while still maintaining the separation of the culture media underneath.

Conclusions: This dynamic culture system contributes to diminishing the time of preparation of complex tissues or multi-tissues and prolonging the viability and use of in vitro and ex-vivo tissues being, therefore, a valuable tool for drug discovery, personalized medicine, and cancer development studies.

Acknowledgments: Consolidator Grant Project “ECM_INK” (ERC-2016-COG-726061).

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keywords: bioreactor,skin,ex-vivo

S10-2
Biofabricated Tissues and Organs for Clinical Impact
Room: S1
(30 Jun 2022, 11:00 - 12:30)

Conveners:
Laura De Laporte

73296309639

WEAVING A COMPLIANT TISSUE-ENGINEERED VASCULAR GRAFT FROM CELL-ASSEMBLED EXTRACELLULAR MATRIX YARN

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Introduction: Vascular grafts are implanted daily, whether it is as leg or coronary bypasses or as arteriovenous shunts. Autologous blood vessels are the gold standard but have limited availability while synthetic materials are prone to thrombosis, intimal hyperplasia and infections. To overcome these limitations, our team produced a biological Tissue-Engineered Vascular Graft (TEVG) woven from yarn of Cell-Assembled Extracellular Matrix (CAM). This textile-based approach is very versatile because it gives fine control over the geometrical and mechanical properties of the TEVG. The goal of this study is to establish how changes in production parameters (e.g.: yarn count, yarn density, etc.) affect the properties of the TEVG (e.g.: mechanical properties, wall thickness, surface waviness, etc.).

Methodology: CAM sheets were produced by sheep dermal fibroblasts seeded in 225 cm2 flasks and cultured in DMEM/F-12 with 10% FBS and 0.5 mM Na L-ascorbate. Threads were cut with a custom motorized device composed of rolling blades spaced at the desired width (5 mm). Woven grafts were assembled on a circular loom and composed of a series of longitudinal threads, called "warp", and a circumferential one that spiraled along the length of the vessel, the "weft". The latter was made of two threads twisted together at 5 revolution/cm. The effects of yarn thickness and warp count (number of longitudinal threads) were tested. The influence of weft thread production parameters and its tension during weaving were also studied. Transmural permeability, suture retention strength, compliance and burst pressure were evaluated. Geometrical properties including TEVG inner diameter, wall thickness and graft lumen surface profile were assessed macroscopically and by X-ray microtomography. For each condition, n=3 TEVGs were woven.

Results: A lower yarn thickness decreased wall thickness and suture retention strength. A lower warp count had the same effect on wall thickness, while the compliance increased linearly with a decrease in warp count. In addition, the surface profile, which may have an impact on cellular infiltration and blood compatibility, was influenced by both parameters. Narrower weft ribbons decreased weft diameter which resulted in thinner walls and TEVGs with lower strength. Finally, a lower tension in the weft during weaving resulted in a significantly higher compliance while burst pressure was decreased.

Conclusion: We have demonstrated the influence of a number of parameters on the geometric and mechanical properties of a TEVG woven from CAM yarn. Investigation of the influence of weft twist is underway to improve our control over the properties of the TEVG, focusing on increasing its compliance. These results are helping to build a toolbox that will allow the production of the TEVG with the most relevant properties for implantations as an arteriovenous graft in sheep.

keywords: Vascular graft, weaving, cell-assembled extracellular matrix

41883625124

EXPLORING SHAPE VERSATILITY ON ALL-AQUEOUS PROCESSING FOR CELL ENCAPSULATION

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Cell encapsulation in biomaterial microcompartments is a useful tool to deliver protected cell cargo into defective tissues. However, the processing of micrometric structures is often dependent on the use of typical emulsion agents, including oils and organic solvents. Those are often related with poor cell viability, or require complicated washing procedures to retrieve oil-free cell-laden materials. All-aqueous processing methodologies arose as promising strategies to process biomaterials with high cytocompatibility. Aqueous two-phase systems have been used as surrogates of classical water/oil-based emulsions to enable phase separation to enable the formation of, for example, microparticles. Experimental approaches similar to the ones widely explored for classical emulsion systems for biomaterial fabrication – including their adaptation into electrospraying or microfluidics-based techniques - have led to the formation of mostly spherical structures, which mostly comprise either continuous biomaterial beads, or hollow capsules obtained upon interfacial reactions. Concerning the latter, the interfacial complexation of polyelectrolytes at the interface of millimetric and micrometric droplets has enabled the cytocompatible encapsulation of mesenchymal stem cells along with adhesive microparticles [1]. However, unlike the preparation of thermodynamically stable spherical objects at the interface of emulsions, the preparation of soft compartments with other shapes has proven challenging. Although previous attempts have been made to fix the shape of objects at the interface of aqueous emulsions, structures capable of being handled outside of the emulsion interface could not be obtained.

We here report an advance on the all-aqueous fabrication of continuous, non-leaky self-standing biomaterial tubes, capable of withstanding cell adhesion on their walls. Another versatile aspect of materials prepared using all-aqueous emulsions is related to the formation of multicompartment complex hollow structures assembled upon the establishment of specific processing parameters. Those were useful in spontaneously separating micrometric objects in different compartments enabling, for example, the encapsulation of viable cells in specific locations of multiwalled structures.

The versatility achieved with the use of all-aqueous emulsions may be used to enable the fabrication of advanced biomaterials that may combine tailored release profiles of bioactive molecules with the exact positioning and protection of cells.

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Acknowledgements:

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keywords: all-aqueous fabrication; cell encapsulation; printing

83767252008

MICROFLUIDIC PRODUCTION OF IMMUNOPROTECTIVE ENZYMATICALLY CROSSLINKED POLYETHYLENE GLYCOL-TYRAMINE MICROGELS FOR BETA-CELL REPLACEMENT THERAPIES

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INTRODUCTION:
Transplantation of non-autologous β-cells is currently regarded as a promising therapy for the treatment of type 1 diabetes, caused by massive β-cell destruction, consequently resulting in insulin shortage. To evade the host's immune responses, new materials are being developed to encapsulate and shield implanted β-cells. Several immunoprotective material formulations have been developed, but their clinical translation is challenged by their impermanent nature, development fibrous capsules upon implantation[1], which is insufficient to produce therapeutical signifficance[2]. Additionally, such materials should be capable of inhibiting the diffusion of large immune molecules (i.e., IgG) whilst enabling diffusion of small molecules (i.e. Insulin and glucose) in a semi-permeable fashion[3]. In this work, we have developed non-immunogenic, immunoprotective, and enzymatically crosslinked, hollow, polyethylene glycol-tyramine (PEG-TA) microgels for β-cell delivery therapies.

METHODS:
Polyethylene glycol 8 arm – tyramine (PEG-TA) conjugates were synthesized in a two-step reaction and optimized for enzymatic crosslinking using horseradish peroxide and non-cytotoxic levels of hydrogen peroxide[4]. Droplet generation was optimized for production of micrometer-sized hollow PEG-TA microgels laden with beta cells using microfluidics[5]. Hollow microgels containing MIN6 pancreatic cells were extensively characterized in vitro based on variables such as cytocompatibility, cyto-immunity, permselectivity, and glucose responsiveness. Furthermore, in vivo performance was assessed by implanting diabetic STZ-mice to investigate immunoprotectiveness of the implant and its ability to re-establish normoglycemia.

RESULTS & DISCUSSION:
Consistent production of ~120-150 μm hollow microcapsules with ~20 μm shell thickness was achieved and characterized. Immunoprotection was confirmed by exposing cell-laden gels by

absence of diffusion of IgG-FITC molecules into the microgels and by co-culture with natural killer NK-92 cells without inducing cell death.
Encapsulated cells were shown to sustain viability and capable of secreting insulin in vitro over a period of one month without showing significant shell burst. Immunological assessment by multiplexed ELISA analysis on live blood immune reactivity was performed, demonstrating little pro-inflammatory cytokine release in response to microgel presence, confirmed by gene expression analysis and immunostainings of inflammatory markers.
Intraperitoneal implantation of β-cell laden microgels in diabetic mice showed restoration of normoglycemia within the first 5 days and good tissue integration, with histology revealing alive aggregates at the time of sacrifice (14 days)

CONCLUSION:
Consistent high throughput microfluidic production of immunoprotective PEG-TA microgels was achieved. The produced microgels revealed good suitability for shielding and delivering non-autologous beta-cells within a living host.

ACKNOWEDGEMENTS: Financial support was received from the European Research Council (ERC, Starting Grant, #759425) and JDRF.

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keywords: Microfluidics, Type I diabetes, Microgels, Beta-cell transplantation, Immuneprotection

83767208404

TISSUE ENGINEERED GRAFT FROM HUMAN ADIPOSE-DERIVED STEM CELLS FOR PHALANX CONSTRUCTION IN CHILDREN WITH SYMBRACHYDACTYLY

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Symbrachydactyly is a rare congenital upper limb anomaly, that occurs in 1/30,000- 1/40,000 live births resulting in children born with short boneless fingers. Nowadays, these pediatric patients are treated with phalangeal bone transfer from the foot. However, morbidities are occurring at the donor site which result in unstable toes with significant disfigurations that worsen with the child growth.

In this project, we used a developmentally-inspired strategy to engineer osteogenic grafts for phalanx reconstruction via endochondral ossification (ECO). Human adipose-derived stem cells (ASC) isolated from the stromal vascular fraction (SVF) were seeded into collagen sponges and exposed to chondrogenic and hypertrophic factors to generate in vitro clinically-pertinent osteogenic grafts (100-200 mm3) in the form of hypertrophic cartilage templates (HCTs).

Specifically, we evaluated in vitro the impact of (i) the cell source (freshly isolated SVF cells or expanded ASCs), (ii) the scaffolding material (collagen type I sponge crosslinked or not), (iii) the cell seeding density (3x105 to 3x106 cells/construct) and (iv) the duration of exposure to chondrogenic (3 to 5 weeks) and hypertrophic factors (1 to 2 weeks) on the maturation of the HCTs generated. The mineralization (by alizarin red staining) and the cartilage maturation (evidenced by the cell morphology and the uniformity and intensity of glycosaminoglycan (GAG) revealed by Safranin-O staining) of these HCTs were evaluated on histological sections. Next, the bone forming capacity of these HCTs was assessed in vivo in an ectopic nude (immunocompromised) mouse model for up to 12 weeks, reflecting the clinical scenario of phalangeal soft tissue pocket

In vitro, we were able to generate HCTs of pertinent clinical sizes. As expected, the maturation of the HCTs was dependent on the duration of exposure to chondrogenic and hypertrophic factors. In addition, we observed that the cartilage formation was obtained more rapidly when using freshly isolated SVF cells rather than expanded ASCs. Overall, the best in vitro outcome was obtained for the crosslinked collagen sponge loaded with SVF cells at the highest cell density.

In vivo, we observed that the bone formation was correlated with the degree of hypertrophic cartilage maturation. Interestingly, even the HCTs that had very limited cartilage at the time of implantation were capable of generating bone once implanted, suggesting that the cells primed in vitro are capable of forming cartilage before the bone remodeling occurs in the early stages of the implantation. Similarly, across all the conditions tested, the quantity of bone tissue obtained

in vivo was superior to the quantity of cartilage tissue obtained in vitro. Finally, while inferior to the freshly isolated SVF cells-based constructs, ASCs-based constructs remained capable of generating clinically pertinent bone tissue in vivo.

Taken together, these results demonstrate the feasibility of using SVF cells or expanded-ASCs to generate osteogenic grafts of pertinent clinical size in the context of symbrachydactyly. Moreover, despite the limited amount of donor-tissue available in pediatric patients, the data obtained for the expanded ASCs suggest that an autologous approach to generate osteogenic phalanx grafts for children born with symbrachydactyly would remain possible.

keywords: Endochondral ossification, human Adipose-derived Stem Cells, Stomal Vascular Fraction cells, Bone tissue engineering, Pre-Clinical study

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BIOFABRICATION OF TUMOR MODELS THAT MIMIC THE TUMOR MICROENVIRONMENT USING EXTRUSION BIOPRINTING

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Cancer, as a cause of death, is only surpassed by cardiovascular diseases. Thus, it is critical to achieve progress in its treatment and prevention. Given the complexity and heterogeneity of cancer, various therapeutic targets are being investigated, including components of the tumor milieu. The tumor microenvironment (TME) consists of several types of cells (vascular cells, tumor-associated fibroblasts, immune cells, mesenchymal stem cells, and adipocytes) embedded in extracellular matrix soaked by interstitial fluid rich in soluble factors secreted by cells [1]. Increasing evidence indicates that tumor progression depends on the interaction between the tumor and its microenvironment and that the effectiveness of anti-cancer therapies is modulated by changes in the TME [1-3]. Therefore, extensive research efforts are devoted to investigating the spatial organization of the native TME and to build in vitro models of the TME using three-dimensional (3D) bioprinting [4] and tissue-on-a-chip techniques [5].

In this work, we report the 3D bioprinting of avascular structures that recapitulate several features of the TME [6]. In our model, the tumor is represented by a hydrogel droplet uniformly loaded with breast cancer cells, whereas the microenvironment is modelled by rings of hydrogel loaded with peritumoral cells: tumor associated fibroblasts and peripheral blood mononuclear cells. The tumor cells used in our experiments came from a commercial cell line (SK-BR-3), while the peritumoral cells were obtained from breast cancer female patients in different carcinoma stages. The cells were embedded in CELLINK Universal Bioink at concentrations of 1 million cells per milliliter, and the tumor models were fabricated using extrusion bioprinters (INKREDIBLE and BIO X, CELLINK, Sweeden). For the optimization and precise control of the printing process, we developed in-house Python scripts able to generate the G-code instructions for the two bioprinters based on the geometries of the digital models. Our workflow was designed to permit the subsequent bioprinting of desired constructs on multiwell plates of different dimensions. After two weeks of in vitro culture, histological cryosections of the tumor models showed that the hydrogel used in this study was appropriate for sustaining cell growth and proliferation. When tumor models were implanted subcutaneously, in the dorsal region of CD1 Nu/Nu immunosuppressed mice, within 28 weeks in vivo they became vascularized and grew about 5 times in diameter.

In conclusion, our work presents a reliable methodology for building models of the TME using extrusion bioprinting. Such models can be used for fundamental research or, if built from

patient-derived cells, for testing the effectiveness of anti-cancer therapies, thereby contributing to personalized treatment plans.

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keywords: extrusion bioprinting, breast cancer models, tumor associated fibroblasts, peripheral blood mononuclear cells

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TISSUE ENGINEERED GRAFT FROM HUMAN ADIPOSE-DERIVED STEM CELLS FOR PHALANX CONSTRUCTION IN CHILDREN WITH SYMBRACHYDACTYLY

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keywords: Endochondral ossification, human Adipose-derived Stem Cells, Stomal Vascular Fraction cells, Bone tissue engineering, Pre-Clinical study

62825428926

AN INNOVATIVE IN VITRO GUT-ON-A-CHIP MODEL TO INVESTIGATE INTESTINAL MICROBIOTA IMPACT ON BRAIN FUNCTIONALITY

Francesca Donnaloja (Politecnico di Milano, Milano, Italy), Luca Izzo (Politecnico di Milano, Milan, Italy), Marzia Campanile (Politecnico di Milano, milan, Italy), Simone Perottoni (Politecnico di Milano, milan, Italy), Lorenzo Sardelli (Politecnico di Milano, milan, Italy), Lucia Boeri (Politecnico di Milano, milan, Italy), Diego Albani (IRCCS - Istituto di Ricerche Farmacologiche "Mario Negri", milan, Italy), Carmen Giordano (Politecnico di Milano, Milan, Italy)

INTRODUCTION
The role of gut microbiota in neurodegeneration is becoming a very interesting topic nowadays, and innovative in vitro and in vivo tools are becoming increasingly a need to help in dissecting the biochemical pathways involved in microbiota-brain interaction. An innovative engineered multiorgan-on-a-chip platform able not only to mimic microbiota-gut-brain connection but also to reproduce in vitro biological and mechanical stimuli to represent the so-called microbiota-gut-brain axis (MGBA) is the aim of our ERC project "MINERVA". MINERVA platform is based on a 3D printed device compatible with commercially available tissue culture inserts and characterized by optical accessibility and capability to be connected to other devices. In the present work, we present our preliminary results related to the development of the gut epithelium modelling unit of our MGA engineered platform.

METHODOLOGY
Computational fluid dynamic simulations were performed with the software COMSOL Multiphysics® testing different flow rate values to obtain suitable oxygenation and shear stress values.
Biological validation was performed using human Caco2 cells seeded on collagen-coated inserts. After 7 days in static conditions, we assembled the seeded inserts into MINERVA devices under perfusion for other 7 days. As control we used seeded inserts maintained in static culture for 14 days. Cellular viability was tested by MTS test, FITC-dextran permeability and Trans-epithelial electrical resistance (TEER) evaluation. To assess cell layer morphology and maturation, immunofluorescence tests were performed with anti-mucin, anti-occludin, FITC-phalloidin and Hoechst dyes.

RESULTS AND DISCUSSION
From computational simulations we set flow rate at 30μL/min to guarantee oxygen supply and suitable shear stress value.
While MTS test showed no difference in terms of viability between static and dynamic condition, TEER values of static samples showed significant differences in line with apparent permeability (Papp) value, that resulted enhanced in dynamic condition.
Occludin and mucin reactivity are comparable between static and dynamic samples and their expression changes along the villus vertical axis. Morphological analysis confirmed mature cell layer formation in both static and dynamic samples with significantly higher villi in dynamic condition.

CONCLUSION
The selected flow rate set to mimic optimal physiological condition, maintained cell viability, promoted villi height and induced TEER value comparable to those of in vivo human intestinal epithelium [1]. Lower TEER values and higher permeability in dynamic condition are coherent

with immunofluorescent data that confirmed mature cell layer formation characterized by lower cellular differentiation near to the villus base, likely required to guarantee villi turnover [2]. Overall our results confirm MINERVA device suitability for gut epithelium modelling in MINERVA MGA multi-organ platform.

ACKNOWLEDGMENT
MINERVA project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (Grant Agreement N° 724734).

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keywords: microbiota-gut-brain axis, neurodegeneration, millifluidic device, gut-on-a-chip, in vitro model

83767214766

AXIALLY VASCULARIZED MANDIBULAR REGENERATION, A JOURNEY OF THOUSAND MILES TO IMPROVE PATIENTS' SMILES

Ahmad Eweida (University of Alexandria, University of Heidelberg, Alexandria (Egypt), Ludwigshafen (Germany), Egypt), Mohamed Morsi Abdeldayem (University of Alexandria, Alexandria, Egypt), Ulrich Kneser (University of Heideberg, Ludwigshafen, Germany)

Introduction:
Axial vascularisation of tissue constructs is essential to maintain an adequate blood supply for a stable regeneration of clinically relevant 3D tissues. In the last 10 years, our research group has been investigating the efficacy of the arterio-venous loop (AV loop) as a model of axial vascularisation. The versatility of the AV loop could be demonstrated in various small and large animal models even after applying high doses of ionizing radiation comparable to those applied for adjuvant radiotherapy after head and neck cancer.

Methodology and Results:
We will report about our experience with axially vascularized tissue engineering constructs in the last 10 years including the in vivo irradiation of the rat and rabbit implantation chambers as well as the mandibular goat model. Furthermore, our most recent results showed a correlation between a state of controlled hypoxia inside the AV loop-constructs and its ability to attract progenitor cells from the systemic circulation. We will also present the early results of our recent clinical trial (NCT04001842) regarding axially vascularized mandibular regeneration of extensive defects after recurrent Ameloblastoma otherwise requiring free flap reconstruction.

Conclusions:
Axially vascularized tissue engineering constructs represents a convenient method to reconstruct extensive mandibular defects otherwise requiring free bone transfer. Despite their proven versatility after irradiation in pre-clinical trials, human application after cancer ablation and irradiation remains a major challenge that should be further investigated in well-designed clinical trials.

keywords: axial vascularisation, clinical trial, mandibular reconstruction

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ENGINEERING THE BIOARTIFICIAL FILTRATION UNIT IN A KIDNEY USING POLYHYDROXYALKANOATES

Syed Mohammad Daniel Syed Mohamed (University of Sheffield, Sheffield, United Kingdom), Gavin Welsh (University of Bristol, Bristol, United Kingdom), Ipsita Roy (University of Sheffield, Sheffield, United Kingdom)

Introduction
Kidney failure happens due to two conditions; acute kidney injury (AKI) and chronic kidney disease (CKD). These lead to the deterioration of the glomerulus, the filtering unit in the kidney, leading ultimately to end-stage renal failure (ESRF). [1] Current treatments for ESRF, haemodialysis and kidney transplantation are inadequate since haemodialysis replaces filtration but not all other kidney functions, and transplantation is limited due to the shortage of donor organs. Therefore, to provide new treatment options, we are using a highly biocompatible bacterial polymer called Polyhydroxyalkanoates (PHAs) as a scaffold material for human kidney cells to develop a bioartificial glomerular filtration barrier.

Methodology
Bacterial fermentation was carried out to produce Polyhydroxyalkanoates using a selected bacterial strain, fed by specific fatty acids to produce a medium chain-length polyhydroxyalkanoate (mcl-PHA). The polymer produced was thoroughly characterised using; Gas Chromatography (GC) for monomer composition, Gel Permeation Chromatography (GPC) for polymer molecular weight, and Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC), both for thermal properties. Two types of glomerular cells, conditionally immortalised human podocytes (CIHP) and conditionally immortalised glomerular endothelial cells (ciGEnC), were used to test the biocompatibility of the mcl-PHA using the resazurin assay [2]. In addition, live dead assays were carried out using calcein green and ethidium bromide. The mcl-PHA was subjected to 3D printing (Fused Deposition Modelling) to engineer a kidney bioartificial filtration barrier. The CIHP and ciGEnC cells were separately bioprinted, using alginate [3] as the encapsulating agent, onto the polymer scaffold to introduce spatial separation.

Results
Mcl-PHA was produced with a yield of around 60 g of polymer per 100 g of dry cell weight (~60% dcw). [3] The polymer monomer composition revealed a higher 3HO percentage than 3HD monomers, with trace amounts of 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HHx) monomers. From the GPC, the average molecular weight, Mw was around 100,000 g/mol. Tensile testing confirmed the elastomeric nature of the polymer, and a low melting temperature (Tm) enhanced the printability of the polymer. Cytocompatibility test showed for the first time that mcl-PHA was highly compatible with both the glomerular cells, CIHP and ciGEnC, comparable to tissue culture plastic (TCP). However, the cells are slightly less viable with alginate as an encapsulation agent, which needs improvement in enhancing the bioactive properties of the encapsulation agent.

Conclusion
This work aims to bio-mimic the human glomerulus to ultimately develop a bioartificial kidney using a tissue engineering strategy and bioprinting. In addition, we have shown that the polymer supports the adherence and growth of human glomerular cells despite the well-known

hydrophobicity. In future, the bioartificial filtration barrier developed using the combination of the mcl-PHA along with glomerular cells will be assessed for its' ability to conduct haemofiltration, eventually, leading to the development of a complete bioartificial kidney.

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keywords: Polyhydroxyalkanoates, tissue engineering, kidney, bioartificial organ, 3D printing

31412720605

TOWARDS THE DEVELOPMENT OF A GELMA-BASED ORGANOTYPIC HUMAN SKIN MODEL USING A CUSTOM-MADE BIOREACTOR

Zahara Saoud Faria Eltayari (ICVS/3B's, Guimarães, Portugal), Luca Gasperini (ICVS/3B's, Guimarães, Portugal), Alexandra Marques (ICVS/3B's, Guimarães, Portugal)

INTRODUCTION: Collagen I has been the gold standard material to generate many in vitro tissue models, including organotypic skin [1]. The rate of collagen remodeling by fibroblasts incorporated in the dermal part of the model, leads to significant dimensional changes during its development, which can be associated to additional hurdles when cultured within dynamic culture systems. These ones, are particularly relevant for skin tissue due to its multilayered nature, by providing the necessary fluid flow for tissue development and interlayer communication for biofunctionality mimicry. Thus, in this work, a different biomaterial, gelatin methacrylate (GelMA), is proposed to validate a dimensionally stable organotypic skin model developed and maintained in a custom-made bioreactor [2], generating a dynamic in vitro testing platform.

METHODS: Two solutions of 5% and 7.5% (wt/vol) GelMA were prepared and crosslinked for 30 seconds under 7.2 mW/cm2 UV intensity [3]. Rat tail collagen I hydrogels were prepared from 0.26% (wt/vol) solutions that were polymerized for 2 hours at 37°C. Human dermal fibroblasts (hDFbs) were encapsulated in the hydrogels at a density of 2.3×105 cells/ml and cultured in α-MEM medium up to 14 days. The elastic (G') and viscous modulus (G'') of the hydrogels were measured using a rotational rheometer and an uniaxial compression test allowed the determination of the compression modulus of the hydrogels. Cell viability was assessed after Calcein/PI staining and ECM deposition (collagen type I and laminin) was analyzed through immunocytochemistry.

RESULTS: The G' of 5 and 7.5% GelMA hydrogels did not significantly vary along the culture, presenting respectively mean values of 0.9kPa and 1kPa, independently of the day of culture. Likewise, the compression modulus did not vary, being within the range of 5kPa and 18kPa, respectively for the 5% and 7.5% GelMA hydrogels. In opposition, the G' mean value of the collagen I hydrogels, increased from 0.5 to 1.2kPa from day 1 to day 14 of culture. The compression modulus, also showed significant differences at day 14, being collagen about 3-fold stiffer than GelMA hydrogels. Moreover, the shrinkage ratio of GelMA and collagen hydrogels was circa 20% and 83%, respectively. Independently of the mechanical properties, GelMA didn't negatively affect cellular viability being also capable to support ECM deposition.

CONCLUSION: From the results achieved so far, the use of GelMA as a substitute of collagen for generating organotypic skin models has demonstrated to possess several potentialities that surpass the struggles associated with the shrinkage of collagen. Future experiments will focus on the incorporation of human keratinocytes (hKCs) on the top of the constructs, to replicate the epidermal compartment and the in vitro dynamic culture of the full skin equivalent will take place in the recently developed sandwich-like bioreactor.

ACKNOWLEDGEMENTS: Financially supported by ERC Consolidator Grant ERC-2016-COG-726061.

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keywords: organotypic skin, in vitro model, GelMA hydrogels, mechanical properties, dynamic culture, bioreactor

94238148339

LASER-BASED SUBTRACTIVE MANUFACTURING FOR TISSUE ENGINEERING

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Introduction
linical translation of tissue engineering-based therapies is currently limited by the difficulty in inducing essential vascularisation for tissue viability after transplantation. Thick and metabolically demanding engineered tissues require a defined microvascular network to provide sufficient nutrient and gas exchange. Laser ablation has emerged as a promising technology to fabricate custom-made perfusable microfluidic channels that mimic capillary beds and aid both anastomosis and vascularization of tissue engineered constructs. So far, the proposed laser-driven methods use expensive laser systems or involve heavy in house customization. In this work, we developed a multistep patterning method to precisely create hierarchical vascular trees using a commercial, affordable and widely available 355 nm laser ablation system.

Methodology
n order to design physiologically relevant capillary networks that consider tissue geometry, physical constraints, and structure stability, vascular trees were generated using a constrained constructive optimization-based method [1]. More particularly, vascular trees were generated using Accelerated Constrained Constructive Optimization as arterial/venous matched pairs meeting at simple anastomoses. Batch optimization was used to minimize a combination of network volume and pump work, with post-build bifurcation asymmetry correction. Inter- and intra-network collisions were resolved, including padding to ensure vessel spacing. Vessels were smoothed and new collisions resolved before export. A Zeiss/Rapp-Opto commercially available laser ablation system was then used. A slicing and tiling algorithm was developed to bridge the gap between 3D CAD model and laser software specific formats. Also, an optimization of the working parameters of laser manufacturing tools (e.g., beam intensity, z-step, overlap, etc.) was required to precisely reproduce the 3D CAD model within a diversity of low stiffness hydrogels.

Results
Ablated features were sliced, imaged and measured through light microscopy. Channels were perfused with 2μm fluorescent beads or injected with commercial silicone rubber and were assessed through confocal microscopy or micro computed tomography, respectively. Complete ablation and formation of open lumen were achieved. Networks of different dimensions created through constrained constructive optimization– including networks larger than the objective were successful recreated. Precise control down to 10μm in resolution was also reported.
Conclusions

In conclusion, this work provides an efficient tool to create customized hollow networks within transparent hydrogel scaffolds in an automated manner. The resulting vascular trees can be used to obtain capillary beds for tissue engineering applications and the development method can be adapted to a multitude of other bio- inspired systems.

Acknowledgements
EU Horizon 2020 research and innovation programme under the ERC grant CapBed (805411), IF/00347/2015 and FCT (S. Queirós, CEECIND/03064/2018).

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keywords: Laser ablation, perfusion, tissue engineering, vascularization

S11
Biofabrication using extrinsic fields
Room: S3 B
(29 Jun 2022, 15:30 - 17:00)

Conveners:
Tiziano Serra

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ULTRASOUND-BASED ASSEMBLY OF TISSUES AND BIOMATERIALS

James Armstrong (University of Bristol, Bristol, United Kingdom)

My research group is focussed on developing new bioengineering technologies that can provide control over the assembly of biomaterials and tissues. I have a long-standing interest in ultrasound: waves of pressure that can interact with living and non-living matter to instigate a range of chemical, physical, and biological processes. First, I will discuss a technology that uses high-frequency ultrasound standing waves to rapidly and remotely pattern living cell populations into tuneable geometric arrays. I will explore our progress in using biomaterials to encapsulate these cellular assemblies and show that these patterned biomaterials can be used to engineer a range of tissues displaying anisotropic structure, which in certain cases leads to directed functional processes. I will also discuss another ultrasound-based technology: the use of low-frequency ultrasound to trigger molecular processes, such as enzyme catalysis and hydrogelation. I will explore the design of this modular system and describe how this new technology opens up new opportunities in tissue engineering and regenerative medicine.

keywords: ultrasound, biomaterials, tissue engineering

94238111005

HIGH-RESOLUTION TWO-PHOTON POLYMERIZATION OF ENGINEERED CELL MICROENVIRONMENTS FOR FUNDAMENTAL NEURO-MECHANOBIOLOGY AND BRAIN CANCER PROTON RADIOTHERAPY

Angelo Accardo (Delft University of Technology, Delft, Netherlands)

Introduction
Nowadays, great attention is devoted to the development of 3D in-vitro neuronal models both for fundamental neuro-mechanobiology applications as well as for disease modelling. Typical approaches include either scaffold-free or scaffold-based strategies. Although the first ones, based on cell self-assembly mechanisms, lead to the formation of tissue-like structures called neuro-spheroids or neuro-organoids, they often suffer from batch-to-batch variability and development of early-stage necrotic cores. Scaffold-based approaches involve instead the use of manufacturing techniques such as fused deposition modelling, bioprinting and electrospinning. These methods however lack the possibility of creating precise micrometric or sub-micrometric geometries able to guide cell fate. In this presentation, I will highlight two recent investigations where we employed a high-definition light-based technology to fabricate for the first time: 1) 2.5D and 3D nanostructures cultured in presence of primary microglia extracted from the brain of rhesus macaque; 2) 3D engineered glioblastoma microenvironments in the context of proton radiobiology studies.

Methodology
All the 3D structures were fabricated by two-photon laser assisted polymerization (2PP), exploiting the two-photon absorption of near-infrared radiation by focusing infrared femtosecond laser pulses onto an organic pre-polymer material. This non-linear mechanism is tuned in order to induce the photopolymerization of the exposed material in extremely confined volumes of sub-micrometric size. In the first study, primary microglia were derived from isolated brain tissue (white matter) of adult rhesus macaque (Macaca mulatta) donors that were free from neurological diseases and cultured both on flat substrates, 2.5D micro/nano-pillars and 3D micro/nano-decorated scaffolds. The morphology of the microglia was then assessed by immunofluorescence and scanning electron microscopy. In the second study, human glioblastoma (GBM) U-251 cells were cultured on 3D architectures mimicking the brain blood vessel geometry and its vascular branching points where GBM cells naturally cluster and proliferate. The engineered glioblastoma microenvironments were then exposed to different proton radiation doses (2 Gy and 8 Gy) and the amount of DNA damage was assessed by using the fluorescence Gamma-H2AX marker.

Results
The combination of sub-micrometric topographies (close to the dimensions of cell filopodia) and mechanical cues (represented by a low effective shear modulus approaching the stiffness of brain tissue), induced a substantial increase in the numbers of microglia characterized by a ramified resting phenotype as compared to cells cultured on flat stiff substrates, mostly featuring an amoeboid morphology. Concerning the second study, upon proton irradiation, GBM cells consistently showed lower DNA damage in the 3D engineered microenvironments compared to 2D GBM cell monolayers, which correlates with the response of GBM cells in-vivo where a greater radioresistance is observed. We hypothesize that this difference in the formation of the number of foci is directly connected to the differences in terms of cytoskeletal properties, cell-matrix interactions and repair kinetics between 2D and 3D cell culture

configurations.

Conclusions
We demonstrate how 2PP can be employed to create: 1) 2.5D and 3D micro- and nano-structures able to guide the fate of primary microglia towards ramified phenotype; 2) 3D engineered glioblastoma microenvironments, which can be used as a reliable benchmark tool for proton radiobiology.

keywords: Two-photon polymerization, Engineered cell microenvironments, Primary microglia, Brain Cancer, Proton Therapy.

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4D BIOFABRICATION OF NERVE GUIDE CONDUITS USING RESPONSIVE MATERIALS

Neha Tiwari (University of Bayreuth, Bayreuth, Germany)

The current gold standard for peripheral nerve repair is autograft. However, the low availability of nerves and loss of function at the donor site are the major disadvantages associated with this procedure. Thus, to address the limited regenerative capability of the human nerves, nerve guidance conduits (NGCs) fabricated using biocompatible and biodegradable materials has proven to be a potential alternative. Various approaches have been explored so far to develop tubular structures for neural regeneration including bioprinting, self-assembly, micropatterning, electrospinning among others. Multiple reports in literature indicates potential of such NGCs in in vitro experiments, however, they fail to provide axonal outgrowth in in vivo studies. The underlying reason is the limitations associated with 3D printing approaches like low resolution of printing tubular structure, thin walled tubes for permeation of nutrients and waste products, high shear forces needed to print the material and difficulty in fixation of endings of ruptured nerves in the nerve conduits. 4D biofabrication based on fabrication of complex structures using 2D and 3D objects by desired shape transformation with response to external stimuli can provide potential solution to the above-mentioned problems. Furthermore, 4D biofabricated structures can be designed to mimic the human tissues like blood vessels, neural tissues etc. In our group, we have designed and fabricated various shape-morphing systems towards tissue regeneration. We observed that fabrication of fibres using electrospinning technique incorporates high porosity which proved to be beneficial towards fast actuation in addition to better exchange of nutrients and waste products. [1, 2] Making use of our previous expertise, in our current project, we are fabricating NGC using smart materials which is expected to overcome limitations not accessible via state of art technologies. We have fabricated fibrous bilayer which is able to self-fold owing to the non-uniform swelling of the two layers. The inner layer of the fibrous bilayer consists of aligned fibres produced using coaxial electrospinning technique with active biomolecules like growth factors in the core and conductive material in the shell. [3] The shell of the fibres will help in providing electrical stimulation whereas the core will help in growth of nerve cells by sustained release of growth factors upon slow degradation. The thoughtfully designed NGCs using smart materials and advanced techniques have potential to overcome the current limitations associated.

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keywords: nerve-guide conduits, electrospinning, stimuli-responsive materials, 4D biofabrication

94238136546

ENGINEERING DORSAL ROOT GANGLION MULTICELLULAR SYSTEM TOWARDS IN VIVO CROSS EXCITATION FUNCTION

Junxuan Ma (AO Research Institute, Davos, Switzerland), Elisa Marani (AO Research Institute, Davos, Switzerland), Mauro Alini (AO Research Institute, Davos, Switzerland), Tiziano Serra (AO Research Institute, Davos, Switzerland)

Introduction
Chronic back and joint pain has been rated as a top risk factor of disability worldwide. In vitro culture of peripheral sensory nerve, namely dorsal root ganglion (DRG) neurons, is a useful model to investigate pain-associated biology and to discover novel regenerative medicine in terms of pain alleviation. Typical monolayer culture of DRG cells, however, losses the multicellular shape, which may cause a disturbed intercellular communication. For example, the multicellular structure provides the basis of synchronized cross excitation commonly observed in vivo [1]. We used the sound induced morphogenesis (SIM) method to aggregate DRG cells into a multicellular system [2]. Viability and calcium signal synchronization of the DRG cells were evaluated in the sound induced multicellular system.

Methodology
DRG cell line ND7/23 was aggregated using SIM in a 0.5 mg/mL collagen solution with a cell seeding density of 0.2 M cells/ml. Cells randomly distributed in collagen gel without SIM served as control (non-SIM control). The DRG cell viability was analysed using live dead staining. After 2 days of culture, the neuronal discharge was evaluated using calcium imaging (Fluo4) [3]. In each culture, 5000 pairs of neurons were randomly sampled to investigate the synchronization of their calcium signalling which was quantified by the ratio dividing their synchronized calcium event number by total calcium event number (synchronization ratio).

Results
Cells in the SIM-induced aggregates displayed higher viability comparing to those outside the aggregate in the same culture (viability 91.9% vs 50.6%) and cells in the non-SIM control (viability 91.9% vs 77.1%). Higher calcium synchronization was found in the SIM group compared to non-SIM control. The synchronization ratio was elevated from 20.4% to 28.1% comparing SIM to non-SIM control. Interestingly, in the SIM culture, cells outside the aggregate also displayed elevated synchronization ratio (29.9%), indicating that the SIM-induced intercellular communication did not depend on an intercellular apposition. Generation of multicellular systems by patterning primary DRG neurons from large animal is ongoing. Omics studies will be performed to unravel their physiological relevance.

Conclusions
The multicellular system allows a better inter-neuronal communication to form the synchronized neuronal discharge which has been formerly observed in vivo [1]. Thus, our multicellular culture system not only reconstruct the complexity of in vivo morphology but is also important to recapitulate the in vivo function.

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keywords: sound induced morphogenesis, 3D model, dorsal root ganglion, calcium imaging, multicellular system

20941830404

CONTROLLING THE SHAPE OF MICROCAPILLARY NETWORKS IN 3D IN VITRO MODELS THROUGH SOUND PATTERNING

Nicola Di Marzio (AO Research Institute Davos, Department of Health Sciences, Università del Piemonte Orientale (UPO), Novara, Italy, Davos Platz, Switzerland), Anne Géraldine Guex (AO Research Institute Davos, Davos Platz, Switzerland), Mauro Alini (AO Research Institute Davos, Davos Platz, Switzerland), Tiziano Serra (AO Research Institute Davos, Davos Platz, Switzerland)

Introduction:
3D in vitro systems are an envisioned alternative to animal models especially in drug testing for research of antitumor treatments (1). Within native tissue microenvironments, the vascular system supports the physiological organ growth with nutrients and growth factors, but also plays important role in pathological conditions such as treatment-resistant tumor progression. Among other cancer types, malignant pleural mesothelioma (MPM) is an example of the latter implication. Reproducing vascular organization within in vitro models of cancer is therefore highly needed for more reliable in vitro drug testing platforms. Therefore, bottom-up approaches and novel biofabrication methods have been increasingly considered for the creation of functional tissues in a layer-by-layer approach. Sound patterning allows the spatial arrangement of biological materials such as individual cells or spheroids (2). The sound-driven hydrodynamic forces induce contactless condensation of cells within hydrogels into defined and reproducible patterns (3). This fast, mild, and simple process can be applied to assemble endothelial cells into a microcapillary network which could serve as vascularization system for functional 3D models at the centimeter scale.

Methods:
Green fluorescent protein expressing human umbilical vein endothelial cells (gfp-HUVEC), human pericytes (hPC) from placenta were used for sound patterning of the microcapillary network layer in fibrin. The patterning procedure was realized with the sound patterning device (mimiX biotherapeutics, Switzerland). The microcapillary network was characterized with fluorescence microscopy. A proof-of-concept of tumor microenvironment (TME) was realized by adding a heterotypic tumor spheroid to the assembled microcapillary network. MPM cells and human fibroblast were used for the tumor spheroid preparation by spontaneous assembly in low adhesion well plates. The growth of the microcapillary network was quantified from the fluorescence image analysis. Ultimately, the effects of anticancer (Cisplatin, Platinol®) and antiangiogenic (Bevacizumab, Avastin®) drugs on the model were evaluated.

Results:
Sound-patterned microcapillary ring networks were created. The high cell packing density induced by sound into the pattern's line facilitated cell-cell connection to form microcapillary structures with the expression of VE-cadherin and lumen formation. The microcapillary ring pattern had a diameter of 1781 ± 142 μm and a thickness depending on the cell seeding density (100-400 ± 30 μm). The presence of the tumor spheroids induced 100 % more area covered by the network compared to the capillary network cultured alone, and further 50 % increase in presence of anticancer drug and tumor spheroid.

Conclusion:
We demonstrated that a microcapillary network layer can be fabricated by sound patterning technology. Thereby, we created a 3D in vitro model used to assess the crosstalk between

different biological components as proven by drug-induced biological response. Overall, in this study a novel concept for biofabrication of vascularized models is presented as an alternative to overcome present limitations. In subsequent studies, the system will be transferred to a custom-built open chamber to host a centimeter scale multicellular construct, allowing for perfusion of the vascular network.

References:
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keywords: Biofabrication, Sound patterning, Microcapillary network,

52354535444

EFFECT OF SECOND STAGE HEATER ON MEW PROCESSING PARAMETERS

Amit Chandrakar (MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, Netherlands), Lorenzo Moroni (MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, Netherlands), Paul Wieringa (MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, Netherlands)

Melt electrowriting (MEW) technique is a manufacturing technology used to fabricate scaffold with user-oriented design. Main distinctive compared to additive manufacturing technique is its ability to fabricate the diameter of few micrometers to sub micrometers range while maintaining high surface to volume ratio. However, this technique has certain drawbacks, such as inaccurate large volume scaffold and fiber placement, sagging and fiber pulsing behavior, and thermal damage to the material properties.

We introduce a controlled way of modulating fiber formation in MEW by optimized installation of a second heater in the vicinity of the Taylor cone, thus presenting additional processing parameters that help to tune the scaffold design parameters more robustly and mitigate some of the current drawbacks of this technique. The primary function of the second heater is to control the solidification rate of the polymer by increasing the ambient temperature surrounding the nozzle. The study is divided into four different sections. 1) Non-isothermal modelling and simulation using COMSOL are performed to optimize the location of the second heater to the nozzle axis and predict the temperature distribution along the spin line region with varying second heater temperatures. 2) Critical speed and fibre pulsing under different conditions are analyzed to evaluate the effect of the second heater on jet stability. 3) Mechanical testing of the stacked fibres is characterized to model/predict the fusion between layers under different conditions adn at last 4) Spinning of thermosensitive and high molecular weigth polymers are investigated, with a low syringe temperature to mitigate degradation and increasing spin line temperature to facilitate processing.

Overall, with the help of the second heater, we were able to fabricate scaffolds from a high molecular weight medical-grade polycaprolactone, poly L-lactide and PVDF, thus expanding the range of materials processable by this exciting technology.

keywords: Melt Electrowritting, High molecular weight, Thermal degradable, Second heater

94238122328

CELL DENSITY MATTERS: LOCAL CELL DENSITY ENHANCEMENT BY SOUND TO INCREASE THE THERAPEUTIC EFFICACY IN REGENERATIVE MEDICINE

Anne Géraldine Guex (AO Research Institute Davos, Switzerland, Davos, Switzerland), Lisa Amanda Krattiger (Department of Obstetrics, University and University Hospital of Zurich, Zurich, Switzerland and Department of Mechanical and Process Engineering, ETH Zurich, Switzerland, Zurich, Switzerland), Bianca Maria Carrara (Department of Obstetrics, University and University Hospital of Zurich, Zurich, Switzerland and University of Zurich, Zurich, Switzerland, Zürich, Switzerland), Mauro Alini (AO Research Institute Davos, Switzerland, Davos, Switzerland), Martin Ehrbar (Department of Obstetrics, University and University Hospital of Zurich, Zurich, Switzerland, Zürich, Switzerland), Tiziano Serra (AO Research Institute Davos, Switzerland, Davos, Switzerland)

Introduction
Within the scope of personalised healthcare in the field of regenerative medicine, patient-derived cells are key players. Their successful application is, however, often hampered by low cell numbers at the expense of donor-site morbidity and lengthy in vitro expansion. Novel biofabrication methods requiring lower initial cell numbers are therefore timely to address this unmet clinical challenge. In vitro, local cell density enhancement by use of sound induced morphogenesis (SIM) at low frequency of <100 Hz was shown to induce increased microvasculature formation at lower cell concentration than conventional methods.1 Based on this, we are developing cell-hydrogel biografts with local cell density enhancement and evaluate their performance after subcutaneous implantation at the back of nude mice. Our research is driven by the hypothesis that local cell density enhancement can improve the therapeutic efficacy in various clinical scenarios such as anastomosis within wounds or bone formation of non-union fractures.

Methodology
We followed a twofold approach, assessing on the one hand anastomosis of implanted human umbilical vein endothelial cells (expressing green fluorescent protein, GFP-HUVEC) and on the other hand ectopic bone formation of mesenchymal stem cells (MSC). To assess anastomosis, HUVEC and MSC were mixed at a 1:1 ratio and resuspended in PEG-based or Dextran-based hydrogels at a final concentration of 2×106 cells per mL. To assess ectopic bone formation, MSC were resuspended in PEG-based hydrogels at a final concentration of 2×106 or 5×106 cells per mL, with or without BMP-2. Cells were then placed on a custom-made acoustic bioprinter and assembled into distinct patterns at a frequency of 60 Hz. Four cell-hydrogel biografts of approximately 4 x 9 mm2 were implanted at the back of nude mice and harvested after 2 or 8 weeks, respectively. Explants were fixed and either imaged as whole constructs or embedded in paraffin for subsequent histological analysis.

Results
By adjusting formulations of PEG-based and Dextran-based hydrogels, time to gelation was increased from 4 minutes to 7 minutes, which proved essential for successful pattern formation by sound. During a 3-day in vitro culture, endothelial cells assembled into pre-vascular structures of tight cell-cell contacts. The animal experiments were conducted with zero mortality during the time of implantation and no other complications. Microscopic evaluation and visualisation of the GFP signal indicated that HUVEC were retained within the PEG-hydrogel after 2 weeks of implantation and formed a pre-vascular network. Further analysis will investigate anastomosis between the host vasculature and implanted HUVEC. Based on visual inspection,

ectopic bone formation was more pronounced in samples and regions of higher cell density. In future experiments, the extent of bone formation will by quantified by micro-CT, followed by decalcification and histological evaluation.

Conclusions
Our results provide evidence that sound induced morphogenesis is a versatile method to produce cell-hydrogel biografts for subsequent pre-clinical evaluation. We demonstrated that local cell density enhancement by sound requires a lower initial cell concentration than conventional methods to achieve comparable microvasculature structures or local osteogenesis.

References
1 Petta et al., Biofabrication 2021, 13, 015004

keywords: acoustic biofabrication, cell density, vascularisation, osteogenesis

S12
Biofabrication with light-based technologies and high-definition printing
Room: S3 B
(29 Jun 2022, 11:00 - 12:30)

Conveners:
Tiziano Serra; Marcy Zenobi-Wong

41935602166

LIGHT-DRIVEN TECHNOLOGIES TO STEER THE FUNCTIONALITY OF VOLUMETRIC ENGINEERED TISSUES AND ORGANOIDS

Riccardo Levato (Utrecht University, Utrecht, Netherlands)

Organ- and tissue-level biological functions are intimately linked to microscale cell-cell interactions and to the overarching tissue architecture. Advances in biofabrication technologies offer unprecedented opportunities to capture salient features of tissue composition and thus guide the maturation of engineered constructs into mimicking functionalities of native organs. Light-based bioprinting techniques enable superior resolution and ability to generate free-form architectures, compared to conventional extrusion technologies. These rely on the spatio-selective polymerization of a bioresin, a photo-responsive hydrogel laden with cells, in response to user-defined, cell-friendly 2D or 3D light fields. In this lecture, the design of new photoresponsive biomaterials for light based 3D printing will be discusses, together with their application for lithographic, layerwise bioprinting and the most recent advances in the development of layerless volumetric bioprinting techniques inspired by optical tomography, capable of processing cm-scale objects in less than 20 seconds. In particular, applications in musculoskeletal as well as soft (liver) tissue engineering are discussed. Notably, as in light-based printing cells are processed in absence of extrusion nozzles, in a contactless fashion, mechanically fragile organoids can be easily introduced as building blocks in the printing process, and shaped into complex and customized, cm-scale 3D tissue analogues. In this way, the ability of organoids to self-assemble enables to generate multi-scale constructs, in which the engineering of the cellular microenvironment is delegated to the (stem) cells that compose the organoid, leveraging their innate capacity for tissue morphogenesis, while at the same taking advantage from the environmental cues influenced by the macroscale milieu provided by the printed hydrogel. With such nozzle and shear stress-free, highly rapid cell processing approach a variety of hydrogel-based constructs can be assembled into hydrogel-based actuators for potential applications in soft robotics, or as platforms to enhance cell viability and maturation post-printing, including the shaping of large networks of hepatic epithelial organoids into defined 3D perfusable structures which exhibit biosynthetic and metabolic functions. This technology opens up new possibilities for regenerative medicine and personalized drug testing, and for the production of new in vitro models for fundamental biological research.

keywords: Biofabrication, organoids, 3D printing, volumetric additive manufacturing

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LASER-BASED HIGH-RESOLUTION 3D PRINTING AND BIOPRINTING FOR TISSUE ENGINEERING

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Various 3D Printing and Bioprinting approaches have proven useful for tissue engineering applications. The achievable spatial resolution of the most widespread technologies, such as for example extrusion, is usually in the range of hundreds of micrometers, limited by the intrinsic attributes of these methods. However, light-based technologies and in particular multiphoton lithography (MPL) can produce features much smaller than a single mammalian cell [1]. Among other things, it has recently enabled realization of highly porous biodegradable microscaffolds capable of hosting individual cell spheroids [2]. The resulting tissue units can be used for bottom-up self-assembly of larger tissue constructs with very high initial cell density [3]. Furthermore, we have recently demonstrated that it is possible to embed living cells using MPL of photosensitive hydrogels, placing this technology in the domain of high-definition bioprinting, as well as fabrication of microstructures directly inside microfluidic devices [4].
MPL opens exciting perspectives for the engineering of advanced microscaffolds and 3D biomimetic cell culture matrices. In this contribution, our recent progress on MPL development will be presented. Current state of the art, challenges and future perspectives will be discussed.

keywords: High-Resolution 3D Printing, Spheroids, Bottom-up Tissue Engineering, High-Definition Bioprinting, Microfluidics

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HARNESSING MICROFLUIDIC BIOPRINTING TO FABRICATE GRADIENT-LIKE POROUS 3D CONSTRUCTS VIA EMULSION INK DEPOSITION

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The exceptional properties of natural structures with density gradients (e.g. bone, sponges, bamboo) have stimulated the interest in reproducing such complex architectures harnessing biopolymer functionality. However, the possibility to generate a hierarchical structure comprising multiple density gradient has not yet demonstrated, mainly due to the lack of technological advancements in engineering of new emulsion materials and rapid fabrication platforms.

In the current work, we reported the 3D printing of porosity-controlled dextran methacrylate (DexMA) oil-in-water (o-w) emulsions using a microfluidic circuit and a fluid-gel support bath. The fabrication of density gradient scaffolds within a supporting gel overcomes the problems associated with low-viscosity bioink extrusion in 3D printing, supporting density gradient structures that would be otherwise impossible to print in-air. The density gradient was engineered using a flow-focusing printhead. The characterisation of the emulsions demonstrated how the regulation of the continuous and dispersed phases by using microfluidic pumps allowed the controlled and automated tuning of the material final porosity. Therefore, we proved that a higher droplet diameter is obtained by increasing the flow rate of the oil phase with a direct and significant proportionality between the diameter and the volume fraction of the dispersed phase ($p<0.0001$). The rheological characterisation of the emulsions revealed a decrease in viscosity as the applied shear rate increased. The continuous phase of DexMA and Pluronic F-68 exhibited a Newtonian fluid-like trend, while the emulsions presented an increasingly pseudoplastic behaviour with expanding dispersed phase volume fraction.

To show the effectiveness of the developed methodology, we realised complex geometries consisting of porous biopolymer fibres, as well as porous scaffolds with axial (two, four and alternate) and radial density obtain differential regions within a single construct. The inclusion of photo-radical initiators in the outer phase of the inks enabled the crosslinking of the structure, following printing, directly into the supporting fluid-gel medium.

The 3D printed porous scaffolds exhibited high-end mechanical properties and elastic response to applied strains. Furthermore, morphological characterisation allowed the observation of the hierarchical internal porous architecture of the scaffolds using X-ray computed micro-tomography (μCT), scanning electron (SEM) and laser scanning confocal microscopy (LSCM), confirming the ability of the novel bioprinting platform to deposit high-resolution density gradient constructs in 3D.

Moreover, we demonstrated the possibility to print highly complex density gradient structures

(e.g. free-standing stairs, inverted pyramids, hollow structures) with extremely low viscosity using an agarose fluid-gel. Furthermore, we investigated the printing of a combination of materials (DexMA and GelMA; DexMA and nHA) by a multi-inlet flow-focusing printhead, resulting in density gradient structures with hierarchical mechanical properties and swelling ability.

Altogether, this work outlines the potential of combining microfluidics and rapid prototyping techniques with the use of a suspending medium, providing a viable alternative for optimally 3D printing of biphasic systems with low viscosities and controlled densities.

keywords: emulsion, gradient, microfluidic printing, flow focusing

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BOTTOM-UP TISSUE ENGINEERING BASED ON MICROSCAFFOLDS PRODUCED BY HIGH-RESOLUTION 3D PRINTING

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Introduction
While the two most-commonly applied approaches in tissue engineering (TE), namely the scaffold-based and the scaffold-free approach come with individual advantages but also drawbacks, Ovsianikov et al. proposed a third strategy for tissue engineering which combines the advantages of both approaches [1].
We propose here to utilize this third strategy to fabricate millimeter-size tissue constructs by fusion of multiple spheroids encaged within microscaffolds. Our approach offers great perspective in regenerating osteo-chondral tissue in vitro, by loading the microscaffolds with human adipose derived stem cells (hASCs) and differentiating them towards osteogenic and chondrogenic phenotypes.

Methodology
The highly porous structures, inspired by the chemical structure of fullerene (C60) referred to as buckyball (BB), were printed using a custom-built Two-Photon polymerization (2PP) system. The 2PP system consisted of a pulsed femtosecond laser operated at 800 nm, that resulted in writing speeds of 600 mm s-1 at an intensity of 85 mW using a 10x objective. The biocompatible and biodegradable, prepolymer called hexa-acrylate-endcapped urethane-based poly-ε-caprolactone (UPCL-6) [2], in combination with 0.5 wt% of photoinitiator M2CMK served as photosensitive resin for 3D-printing. The use of low-adhesive 96-well plates hosting single microscaffolds in each well allowed seeding (4000 cells/well) of hASCs. After 2 days, spheroid formation within the buckyball scaffold was detectable and spheroids were differentiated towards the osteogenic and chondrogenic lineage for 21 days. After differentiation, the microscaffold-reinforced spheroids, referred to as tissue units (TUs) were harvested and merged together in a custom-made cylindrical agarose mold with a diameter of ~1.8 mm and a height of 8 mm for 7 days. 50 TUs differentiated towards the osteogenic lineage were placed at the bottom of the mold, while 50 TUs differentiated towards the chondrogenic lineage were seeded on top of the osteogenic ones to result in an osteochondral interface.

Results and Discussion
The successful differentiation of the spheroids within the microscaffolds towards the osteogenic lineage was verified by calcium deposition quantification, fluorescent calcein green staining, while the successful chondrogenic differentiation was verified by quantification of sulfated glycosaminoglycans and total protein amount. The formation of larger tissue constructs in the range of several millimeters was possible, using these differentiated spheroid-laden BB as

“building blocks”.

Conclusion:
Our results indicate that this third TE method could be a promising approach with wide applicability, as the microscaffold can be used for instance to tailor the nature of the final tissue. In addition, the utilization of different cell sources or differentiation into further lineages could pave the way towards a variety of different TE applications.

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Acknowledgements:
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keywords: bottom-up tissue engineering, high-resolution 3D-printing, biocompatible materials, biofabrication

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DEFINED-GEOMETRY MICROPARTICLES PRODUCED BY TWO-PHOTON POLYMERISATION FOR SKELETAL APPLICATIONS

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Introduction
Bone graft substitutes are typically provided as ceramic granules. Whilst they have undergone successful clinical implementation, they are not without limitations. These include brittleness, variable resorption rates and a lack of control over the microarchitecture, all of which can lead to poor integration of the graft and fibrous tissue formation at the interface. Porous polymer microparticles can overcome some of these limitations and have seen increased research interest recently due to their potential to deliver cells and therapeutics in a minimally invasive manner. As there is evidence that pore size and shape can be used to control mesenchymal stem cell (MSC) fate, defining the microarchitecture of a particle to include beneficial geometries may be a route by which a reliance on co-administration of exogenous growth factors can be reduced. Tightly controlling microparticle architecture at a length scale relevant to bone cells can only be achieved through high-resolution additive manufacturing techniques such as two-photon polymerisation (2PP). Therefore, here we describe the fabrication of multiple defined-geometry microparticles via 2PP from a range of materials and their subsequent evaluation for cellularisation and osteogenesis.

Method
By cross referencing data from a microarray screen of polymers that promote MSC attachment and the number of acrylate moieties on the precursor monomers, potential photosensitive materials for 2PP were ranked. Processing parameters of the top three candidates were optimised to permit rapid fabrication of complex structures with robust structural integrity. Using designs based on mathematical solids to allow precise definition of geometry, these materials were then fabricated into six different designs of 100 μm diameter defined-geometry porous microparticles, and a solid-sphere microparticle control. To assess amenability to cellularisation, each geometry/chemistry combination (21 total) was fabricated into tessellated 1×1 mm arrays to mimic the close packing of particles that would occur in vivo. Arrays were seeded with three different donors of human bone marrow-derived MSCs (N=3, n=4) and cultured for five days before examination by confocal microscopy. By integrating fluorescence intensity across the height of the arrays, cellularisation could be quantitatively compared.

Results and Discussion
Quantitative analysis of array cellularisation revealed variation in pore shape can modulate cell infiltration even with a constant material chemistry. Particular geometry/chemistry combinations outperformed the solid particle controls, and importantly, certain geometries were observed to have a high degree of cellularisation across all chemistries investigated, indicating an architecture with utility in regenerative medicine. Microparticles which encouraged cell infiltration are currently undergoing screening for synergistic osteogenic effects that may further enhance their performance as bone graft substitutes. In parallel, fabrication of 3D arrays (e.g. 1×1×1 mm) of microparticles for assessment in a small animal model are underway.

In summary, 2PP allows us to incorporate a defined, cell-scale internal geometry within porous polymer microparticles. This work revealed a mathematically definable geometry that promotes MSC infiltration in comparison to a solid polymer microparticle which may be of interest clinically and is currently undergoing further evaluation.

Acknowledgements
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keywords: Mesenchymal stem cells, bone, additive manufacturing, 3D printing, 2PP

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MICROFLUIDICS-ASSISTED BIOPRINTING OF DOUBLE-EMULSION DROPLETS

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Introduction
Microfluidic droplet-based bioprinting offers several advantages over conventional extrusion-based bioprinting methods such as (i) high-precision spatial patterning of the biologics (including cells, molecules, drugs and bioinks) and (ii) ease of their compartmentalization. These advantages, combined with high reproducibility of the generated microdroplets, facilitate high-throughput fabrication of well-defined 3D tissue constructs with complex inner architecture which could be used, e.g., in drug- or biomaterial-screening.

Most of the techniques currently availabe in the literature rely on the use of simple W/O emulsion droplets [1]. In this context, double-emulsion W/O/W core-shell droplets consisting of an aqueous 'core' encapsulated by a (biocompatible) oil 'shell' in an external aqueous phase could offer additional benefits. The shell phase could serve as a selective permeable barrier allowing the transport of small molecules and oxygen from the external environment while ensuring that the cells and bioinks contained in different droplets remain compartmentalized and develop into separate microtissues.

Here, we establish the possibility of printing of single-file chains of double emulsion aqueous core-droplets onto a glass substrate under external aqueous media. This strategy allows generation of ordered arrays of droplets for future use as microfluidic biomaterial- or drug-testing assays. In particular, we also demonstrate printing of hydrogel (GelMA) droplets.

Methodology
Double emulsion droplets are generated using an aqueous solution of GELMA 6% w/v + 0.2% w/v photoinitiator (LAP) as the inner phase, NOVEC 7500 + 3 % PFPE-PEG-PFPE surfactant as the shell/intermediate phase and distilled water as the external phase. The substrate is a glass slide treated with a fluorophilic coting NOVEC 1720.

GelMA droplets are encapsulated in NOVEC 7500 using a microfluidic T-junction micromilled in a polycarbonate chip. The generated droplets are then directed towards a substrate through a 25G needle immersed in an external aqueous media. The spacing between the tip of the needle and the substrate is precisely adjusted (<200 micrometers) to allow immediate deposition of the droplets at the substrate via wetting by the oil phase while leaving enough space for the droplets to remain stable upon extrusion.

Results
We show that the GelMA droplets can be printed onto a substrate in the form of a line of liquid 'cores' encapsulated by a thin oil 'shell' under external aqueous media. The presence of the surfactant-rich oil phase not only prevents coalescence of the droplets but also leads to adhesive capillary forces between them which stabilizes the printed lines. We demonstrate

printing of hundreds of droplets at various substrates and in particular find optimal printing conditions using rough or porous substrates which facilitate rapid droplet deposition (prevent droplet ‘sliding’ at the substrate).

Conclusion(s)
We present a microfluididc-bioprinting platform that could, in the future, serve as a novel tool for high-throughput reproducible production and printing of thousands of compartmentalized microtissues. The technology could be developed towards, e.g., high-throughput biomaterial-screening via incorporating different hydrogel in each droplet followed by the deposition of the droplets into ordered arrays and their long-term culture.

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keywords: Microfluidics, bioprinting, soft-granular matter, double-emulsions

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EFFECT OF LIGHT STIMULI IN VOLUMETRIC BIOPRINTING ON CELL FUNCTIONALITY AT SINGLE CELL LEVEL

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INTRODUCTION. Volumetric bioprinting (VBP) is a recently developed light-based biofabrication method enabling the rapid generation of complex 3D structures within seconds. Short printing times combined with freedom of design allow for the advancement of novel in vitro models and physiologically relevant constructs. However, a more in-depth understanding of the effects of light-based bioprinting techniques is needed. During printing, the bioresin volume is illuminated, triggering the production of free radicals needed for common photocrosslinking reactions (i.e. chain growth and thiol-ene). Albeit safe light doses have been identified, radicals include reactive oxygen species, which concentration needs to be kept below supra-physiological values in the proximity of cells to avoid DNA damage. Moreover, in light-based bioprinting, unreacted bioresin volumes (from the reaction vat) are typically discarded, resulting in the loss of valuable cells. Unraveling the impact of cell-light interactions in bioprinting is key for the design of clinically-viable constructs for biomedical applications. Herein, we investigated the cell response after VBP via single-cell transcriptomics and analyzed stress and health markers. Further, we evaluated the usability of recycled mesenchymal stromal cells (MSCs), retrieved from the excess bioresin in the printing vat.

METHODOLOGY. Viability, metabolic activity, and H2O2 production were assessed upon printing human bone marrow-derived MSCs with 10% wt GelMA and 0.1% wt lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). Casted gels (crosslinked with 365nm or 405nm lamps), and enzyme-crosslinked, non-photoexposed gelatin were used as controls. Samples were printed with increasing light dosages (375-625 mJ/cm2). DNA damage was assessed through immunostaining of apoptosis and double-strand breaks markers (Caspase-3, gH2AX, and TP53BP1). To determine inter-cellular variability and screen in-depth for light-based cell effects, single-cell RNA analyses were performed with a library of ≥75000 target genes. Cells were sequenced without undergoing VBP, 1 hour post-printing, and after 7 days in culture post-printing. Finally, MSCs retrieved from the uncrosslinked GelMA were collected and re-plated after printing, to assess proliferation rate and differentiation ability (osteogenic, adipogenic, and chondrogenic lineages).

RESULTS. Viability was unchanged over seven days of culture for the printed structures, compared to the casted gels (>90%), and higher light dosages correlated with higher metabolic activities. The first assessment of H2O2 concentration showed no significant increase post-printing. No significant DNA-damage was measured with the different markers. Re-plated cells had increased metabolic activity post-printing. Initial results from the single-cell sequencing data have shown a mild activation of stress-related genes, mainly involved in the TNF-a and KRAS signaling pathways, 1 hour post-printing. Results from the sequencing of cells cultured 7 days post-printing will shed light on the effect of the maturation process on their transcriptional state, and on the time-dependent response in the transcriptome.

CONCLUSION. Volumetric bioprinting is a fast biofabrication method that allows for the generation of tissue constructs without hampering cell viability and functionality post-printing. High-throughput, single-cell transcriptomic assays have great potential to elucidate the safety and risks related to bioprinting technologies. These results give valuable insights on cell behavior post-printing, which are needed to develop the next generation of bioprinted in vitro models and patient-specific grafts.

keywords: Volumetric bioprinting, DNA damage, Tissue engineering, Photo-responsive biomaterials, Single-cell sequencing

S13-1
Biofunctionalized surfaces for cellular and tissue engineering
Room: S2
(28 Jun 2022, 11:00 - 12:30)

Conveners:
Rui L. Reis

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BIOMIMETIC SURFACE COATINGS AND HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

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Polysaccharides belong to the most abundant biomaterials on earth. They form structural elements of plant and animal tissues, but many of them have also important regulatory functions towards cells, tissues and organs. Glycosaminoglycans (GAG) and other carboxylated and sulfated polysaccharides possess a bioactivity that is due to their high affinity to a plethora of proteins that belong to insoluble and soluble components of cell environments, but also by direct interaction of these carbohydrates with cell receptors. Hence, making surface coatings or hydrogels from natural or semisynthetic GAG for implants that guide cell behavior towards differentiation to bone or cartilage might be an promising approach ti mimik the natural environment of cells in tissues.
Here, covalent binding mechanisms based on oxidation of pendant hydroxyl groups or introduction of reactive side groups like thiols in monosaccharide subunits permit direct coupling to surfaces or cross-linking mechanisms using Schiff´s base or disulfide bond formation, respectively. Physical immobilization can exploit the inherent charge of these molecules that permits formation of multilayers on surfaces kept together by ion pairing, but also intrinsic cross-linking of activated GAG. Both mechanism can be also used to form 3D structures as in situ gelling hydrogels that permit embedding of growth factors and cells.
Native and oxidized GAGs have been used to prepare multilayer coatings that could promote either osteogenic or chrondrogenic differentiation using chondroitin sulfate (CS)and hyaluronan as polyanions, respectively. Multilayer systems with oxidized CS were also useful as system for controlled presentation of osteogenic growth factor BMP-2. Thiolated CS and chitosan could be also used for redox-sensitive multilayer coatings that change their cell adhesion properties in dependence on disulfide bond formation, which functions as stimuli-responsive system for cell culture. Apart from making bioactive surface coatings both cross-linking mechanisms can be also used to form 3D structures as in situ gelling hydrogels that permit embedding of growth factors and cells.
In conclusion of our studies we could fabricate 2D and 3D systems that are instructive in controlling cell spreading, growth and differentiation of mesenchymal stem and other cells, which will be shown with examples of chondrogenic and osteogenic differentiation of cells.

keywords: polysaccharides, biomimetics, surface modification, connective tissue engineering

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SURFACE FUNCTIONALISED BIOMATERIALS AND NANOSTRUCTURES FOR ADVANCED THERAPIES

Nuno Neves (3B's Research Group / University of Minho, Guimarães, Portugal)

Many biomaterials have been proposed to produce porous scaffolds, nanofibers and nanoparticles for different medical treatments and applications. Systems combining natural polymers and synthetic biodegradable polymers offer particular properties adequate for those demanding applications. Those biomaterial systems can be tailored with enhanced mechanical properties, processability, cell-friendly surfaces and tunable biodegradability. Those biomaterials can beprocessed by melting or solvent routes into devices with wide range of applications such as biodegradable scaffolds, films or particles and adaptable to many other high performance biomedical applications.

Non-woven meshes of polymeric ultrafine fibers with fiber diameters in the nanometer range can be produced by electrospinning. Those meshes are highly porous and have a high surface area-to-volume ratio. Furthermore, they can mimic the fibrous structure of the extracellular matrix of human tissues and can be used as scaffolds for Tissue Engineering (TE). There is a great interest in developing also nanoparticles and hydrogels from those polymeric systems for injectable treatment modalities. All those structures can be used as substrates for specific surface functionalization having fine-tuned bioactivity and biological performance. This strategy enables developing highly controlled devices for exposure, capture and, whenever needed, inactivation of biological biomolecules. Those high-performance devices offer the specificity and local bioactivity that enable to design novel treatment modalities in various disease conditions. This talk will review our latest developments biomaterials, nanoparticles and nanofibre meshes in the context of novel therapeutic applications.

keywords: Biomaterials, Stem Cells, Nanoparticles, Drug delivery, Surface biofunctionalization, Tissue engineering, Advanced Therapies

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INTRODUCING CONTINUOUS MATERIAL GRADIENTS IN OSTEOCHONDRAL CONSTRUCTS VIA A NOVEL EXTRUSION-BASED 3D PRINT HEAD

Ivo Beeren (Maastricht University, Maastricht, Netherlands), Sandra Camarero-Espinosa (Maastricht University, Maastricht, Netherlands), Piet Dijkstra (Maastricht University, Maastricht, Netherlands), Carlos Mota (Maastricht University, Maastricht, Netherlands), Ravi Sinha (Maastricht University, Maastricht, Netherlands), Matthew Baker (Maastricht University, Maastricht, Netherlands), Lorenzo Moroni (Maastricht University, Maastricht, Netherlands)

Osteoarthritis, a degenerative disease of the cartilage and subchondral bone, is becoming more prevalent due to an aging world population. Although some clinical interventions are available, the solutions are often temporary due to fibrocartilage formation in the long term, which has mismatching mechanical properties. Tissue engineers have been designing osteochondral (OC) scaffolds to induce functional, sustainable regeneration of the articular cartilage. An important characteristic of the OC unit is the presence of continuous gradients such as biochemical or mechanical ones1. Additive manufacturing is preferably used to create porous fibrous architectures to host cells for tissue engineering. To introduce continuous property gradients in these scaffolds, it seems straightforward to continuously extrude an incremental ratio of two biomaterials during the printing process. Although successfully extruded continuous gradients have been achieved with hydrogels2, it remains challenging for solid polymers. Therefore, solid constructs often contain discontinuous gradients with 'hard' interfaces, prone to delamination, and moreover a lower degree of biomimicry3. Thus, we aim to design a construct with a continuous material gradient containing specifically addressable groups on the surface to anchor differentiation-inducing peptides. We finally want to create a peptide gradient to gradually direct human mesenchymal stromal cell (hMSC) differentiation, from a chondrogenic towards an osteogenic phenotype, across a construct.
To this end, we synthesized poly(caprolactone) with terminal azide and maleimide groups, which we mixed in a continuous fashion via an in-house developed printhead4. We visualized the continuous material gradient in our constructs with a dye and show control over the gradient distribution. Additionally, mechanical analysis showed that, when comparing a discrete and a continuous gradient, the brittleness of our PCLA is lost in the continuously extruded scaffolds. Furthermore, we successfully modified the surface by reacting complementary dyes on the surface with a density in the 102 pmol/cm2 regime. After a three week cell differentiation study, we observed a strong effect of the differentiation media on hMSC fate. Interestingly, in basic media with our chondrogenic peptide, we observed enhanced (hypertrophic) chondrogenic differentiation in absence of the differentiation factors. Finally, our materials showed good biocompatibility, without severe toxic effects, and abundant tissue regeneration in a subcutaneous rat model.
Overall, we have successfully manufactured a construct with a continuous material gradient via an extrusion-based approach using an in-house developed printhead. Moreover, the continuous gradient construct has more resilient mechanical properties and a modifiable surface, making this fabrication method also interesting for applications beyond OC ones. Our hMSC differentiation study shows promise to influence cell fate in the basic media, but further investigation on the active parameters to achieve larger cell fate differences is required, before moving towards gradual differentiation.

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keywords: Continuous material gradients, additive manufacturing, surface modification, hMSC differentiation

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HIGH-CONTENT IMAGE-BASED PROFILING FOR EVALUATING THE EFFECT OF PEPTIDE COATING EFFECT ON MEDICAL DEVICES

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Introduction

In recent years, biomolecules have been used to functionalize the surface of scaffold materials to support tissue engineering applications. For the functionalized surface for enhancing the cell culture efficiency, peptide coating has been one of the major strategies to provide surface property mimicking the extracellular matrix (ECM). In the past days, the cell adhesion triggered by such surface modification molecules had been mainly understood through the ligand-receptor interactions, such as integrin recognition mechanisms. On the other side, recent mechanobiology studies have revealed that cells are attracted and recognize more varieties of scaffold surface parameters, such as physicochemical properties. Proteoglycan-associated cell interactions are one of such cell-surface interactions. However, compared to the integrin-mediated adhesions, such cell-surface interactions triggered by physicochemical properties have not yet been clarified, since there are too many parameters to investigate. In order to understand the complex combinational effect of physicochemical parameters on the cell adhesion surface, we focused to examine the effect of peptide-coated surfaces, since peptides are functionalization molecules that can be designed in a combinatorial manner. Our research group has successfully obtained several cell-selective adhesion peptides and osteogenesis-promoting peptides by peptide array screening and has been carrying out research on peptide-based materials with high functionality [1,2]. In addition, we have previously reported that amino acids on the surface of materials can change cell adhesion [3]. In this study, we investigated to evaluate the combinatorial effect of peptide and amino acid-coated surfaces by the introduction of high-content image analysis.

Methods

By combining laboratory automation technology, image processing, and peptide surface modification techniques using DOPA, we developed a new image-based profiling platform to evaluate the delicate differences of cell adhesion profiles. On the multi-well plate, we immobilized RGD peptide with and without the coating of single amino acids (20 variations) and created the surface conditions in which the neighboring physicochemical atmospheres are different with the same RGD coated surface. On such combinatorial property design surfaces, we evaluated the cell adhesion and cell extension rates by fluorescent image processing capturing cytoskeleton responses.

Results and conclusions

From our data, we found that the surface physicochemical properties created by amino acid coating drastically changed the RGD peptide functionalization effect, and therefore lead us to find the optimum surface property condition to maximize the peptide effect for cell adhesion. Our results suggest that the control of physicochemical property design can empower and

stabilize the surface functionalization of cell culture scaffolds.

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keywords: Peptide, Laboratory automation, Image processing, Cell adhesion, Physicochemical atmospheres,

83767201967

BFGF-FUNCTIONALIZED POLYISOCYANOPEPTIDE HYDROGEL FOR TISSUE REGENERATION OF THE PELVIC FLOOR

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INTRODUCTION:
Pelvic Organ Prolapse (POP) is characterized by the descendance of the pelvic organs due to weakening of the pelvic floor. Up to 20% of the women get recurrence after surgery, implying that surgical outcomes are poor due to suboptimal wound healing[1]. Tissue engineering has shown great potential in stimulating regeneration by combining cells, biomaterials and biochemical cues. Polyisocyanopeptide (PIC) hydrogels are synthetic, thermosensitive and highly biomimetic, displaying stress-stiffening behavior similar to other biomacromolecules[2]. Furthermore, the PIC hydrogel can be functionalized with growth factors, like basic fibroblast growth factor (bFGF) to further promote cell proliferation and extracellular matrix (ECM) remodeling[3]. In this study, we developed a bFGF-functionalized hydrogel and investigate its wound healing capabilities in vitro.

Methodology:
PIC polymers were synthesized and conjugated with a cell-adhesive peptide GRGDS as previously reported[4]. bFGF was reacted with DBCO-PEG4-NHS and Alexa647-NHS at 3 and 1.5 equivalent respectively in PBS. Next, the bFGF-DBCO was purified over a 10 kDa spin filter and conjugated to the PIC polymer overnight at 4°C. The bioactivity of PIC-bFGF was validated on 3T3 fibroblasts and human adipose-derived stem cells (ADSCs) using the CellTiter-Glo® assay. To assess its wound healing capabilities in vitro, the PIC-bFGF (50 ng/mL), encapsulated with ADSCs was evaluated at day 1, 7, 14 and 28. Cell viability was visualized with a LIVE/DEAD staining. ECM deposition was evaluated by quantifying (Sirius red staining) and visualizing (CNA-OG488) collagen and quantification of elastin (FastinTM Elastin assay).

RESULTS:
Dose-response curves were generated to validate the bioactivity of PIC-bFGF (EC50 = 18.3 ng/mL), showing a 3-fold induction in proliferation which is in line with the positive control whereby bFGF is added soluble to the PIC-RGD hydrogel (EC50 = 3.7 ng/mL). ADSCs (EC50 = 17.9 ng/mL) are highly viable in the PIC-bFGF. Furthermore, total collagen amount significantly increases up to day 7 ($p<0.001$; data not shown) resulting in mature collagen network at day 14. Preliminary data shows no significant increase in total collagen and elastin in the PIC-bFGF versus PIC-RGD yet.

CONCLUSION:
PIC-bFGF is bioactive in both 3T3 and ADSCs. Therefore, the PIC-bFGF is very promising and will be studied to investigate whether wound healing can be promoted in vivo. Further research is ongoing to provide more insight in the promoting effect of PIC-bFGF versus PIC-RGD in ADSCs

regarding ECM metabolism in vitro.

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keywords: Hydrogel, Tissue Engineering, Pelvic Organ Prolapse, basic Fibroblast Growth Factor

73296320484

DEVELOPING BRAIN-TARGETING LIPOSOMES TO DELIVER MESENCHYMAL STEM CELLS SECRETOME FOR PARKINSON'S DISEASE REGENERATIVE MEDICINE

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Parkinson's disease (PD) is a neurodegenerative disease clinically characterized by motor disabilities. Current therapies are not being fully effective. Remarkably, the neuroregulatory molecules secreted by mesenchymal stem cells (MSCs) have been suggested as an alternative therapy. However, direct injection into the brain is the delivery approach that has been used in pre-clinical models. Thus, the main goal of this work was to develop brain-targeting liposomes to deliver the secretome of MSCs and allow a systemic delivery treatment. For that, liposomes were functionalized with lactoferrin (Lf), whose receptors are overexpressed in endothelial cells present at blood brain barrier, as well as in dopaminergic neurons at substantia nigra, in both animal models and PD patients. The particle size distribution, polydispersity index (PDI) and zeta potential of liposomes were assessed by Dynamic Light Scattering (DLS). An in vitro release profile study was performed to predict the in vivo bioperformance of MSC secretome-loaded liposomes. To determine the effect of the liposomes on cell viability, MTS assay was performed using SH-SY5Y cells. The DLS results presented hydrodynamic diameters around

100 nanometers and relatively low PDI values. Liposomes were able to encapsulate the MSC secretome, allowing a sustained release profile. MTS assay demonstrated that liposomes did not induce alterations on viability of SH-SY5Y cells. SH-SY5Y cells were differentiated into a mature dopaminergic neuronal phenotype and exposed to the neurotoxin 6-hydroxidopamine to reproduce an in vitro cell model of PD. Lf-modified MSC secretome-loaded liposomes were able to protect the viability of these cells after neurotoxin exposure. A biodistribution study was performed in mice using Texas Red-labelled liposomes, 5 hours after intravenous administration. The study showed that Lf-modified liposomes were detected on the brain of mice in a higher concentration, when compared to the liposomes that were not functionalized. Remarkably, intravenous treatment of Lf-modified MSC secretome-loaded liposomes were able to improve motor disabilities in a mice model of PD. These results highlight the potential of MSC secretome-loaded liposomes to function as a brain-targeting delivery system therapy for PD.

Comment: Bruno F.B Silva and António J. Salgado share senior authorship

keywords: Parkinson’s disease, Functionalized liposomes, MSC secretome, Regenerative Medicine

31412712444

GUIDED CARTILAGE FORMATION: COVALENT GROWTH FACTOR IMMOBILIZATION ON MELT ELECTROWRITTEN MICROFIBER SCAFFOLDS

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Introduction
Current tissue engineering treatment strategies for end-stage articular cartilage fail to produce long term functional cartilage tissue. Here, melt electrowriting (MEW) is used to fabricate 3D scaffolds with micro-resolution to mimic the structural properties of the native cartilage extracellular matrix[1]. These scaffolds are activated using atmospheric-pressure plasma jet (APPJ), allowing for covalent immobilization of transforming growth factor beta 1 (TGF), an important cytokine for the production and maintenance of cartilage[2], onto the scaffold's microfibers. It is hypothesized these biofunctionalized scaffolds will support differentiation of mesenchymal stromal cells (MSCs) into the chondrogenic lineage and subsequent cartilage-like matrix deposition.

Methodology
Poly-e-caprolactone MEW scaffolds were fabricated using a 3DDiscovery printer (REGENHU), then activated using a computer-controlled APPJ device[3]. Wettability and x-ray photoelectron spectroscopy were used to assess surface chemistry changes. TGF was subsequently immobilized onto the MEW scaffolds by submersion in solution (1 µg/mL). Characterization of protein immobilization was performed using enzyme-linked immunosorbent assay (ELISA) and immunofluorescence detection. In silico modelling was performed to investigate the potential benefit of immobilizing TGF rather than supplying the TGF in the medium. In vitro experiments were performed by seeding equine-derived MSCs into the scaffolds and then culturing for 28 days. The groups investigated included APPJ-treated constructs with (+APPJ +TGF) and without (+APPJ -TGF) immobilized TGF, as well as untreated constructs with (-APPJ +TGF) and without (-APPJ -TGF) TGF supplied through the culture medium. Cartilage-like formation was quantified with dimethyl methylene blue/picogreen assays for glycosaminoglycan (GAG) production and confirmed with histological analysis, including safranin-O and collagen type I & II immunohistochemistry. Matrix deposition was additionally analyzed using compressive testing.

Results
ELISA results confirmed covalent TGF concentration on the APPJ-functionalized scaffolds while immunofluorescently-labelled TGF was detected visually in microfiber scaffolds (following 0.1% Tween20 washing). The APPJ treatment caused increased hydrophilicity of the scaffolds, resulting in efficient cellular infiltration. In vitro analysis demonstrated that GAG production was significantly enhanced in both the immobilized TGF (+APPJ +TGF) and TGF (-APPJ +TGF) in medium groups, compared to the control groups without TGF supplementation (-APPJ +/-TGF). This finding was further validated by the heightened production of GAGs and collagen type II, observed in histological sections. In addition, in vitro and in silico analysis revealed that immobilized TGF on the scaffolds was more advantageous than TGF supplied directly through

the medium. Following the 28-day culture period, the immobilized TGF (+APPJ +TGF) construct group exhibited increased compressive modulus (>3 fold) and GAG production (>5 fold) when compared to the TGF in medium (-APPJ +TGF) construct group.

Conclusions
APPJ-surface treatment facilitated covalent immobilization of TGF onto MEW scaffolds. Immobilized TGF retained bioactivity and promoted the differentiation of MSCs into the chondrogenic lineage. Our results also demonstrate that the new constructs with immobilized TGF support cartilage-like-tissue formation. These findings drive new perspectives for reagent-free, growth factor-functionalized constructs with controllable, high-resolution geometries for guided tissue regeneration.

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keywords: cartilage, melt electrowriting, growth factor delivery, biofunctionalized, atmospheric-pressure plasma jet

S13-2
Biofunctionalized surfaces for cellular and tissue engineering
Room: S3 A
(29 Jun 2022, 11:00 - 12:30)

Conveners:
Rui L. Reis

52354529355

ELECTROACTIVE POLYCAPROLACTONE-GRAPHENE NANOCOMPOSITES COMBINED WITH ZINC IONS TRIGGER MYOGENIC DIFFERENTIATION

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Musculoskeletal tissue engineering (MTE) has proven to stimulate survival and differentiation of myoblasts towards tissue regeneration both in vitro and in vivo (1). In this field, different polymeric biomaterials have been employed to provide a biomimetic environment where cells can proliferate and differentiate into muscle tissue.
Polycaprolactone (PCL) is a synthetic aliphatic biodegradable polymer with remarkable mechanical properties, thermal stability, and controllable degradation. It is also approved by the FDA as a biomaterial for biomedical applications, being used in a wide range of biomedical applications, such as tissue engineering, dental implants, and drug delivery. (2,3, 4). PCL-based muscular grafts are promising tools in MTE (3).
Musculoskeletal tissue is well known for its electrosensitive cells since it is innerved by motoneurons that stimulate muscle myofibers to contract. Therefore, it is of great interest to obtain electrically active cell substrates to assess their influence in cell differentiation with and without external electrical stimulation. Different polymeric nanocomposites with conductive particles have been developed in recent years (5). Graphene (G), a polycyclic aromatic hydrocarbon with excellent conductive properties, has been incorporated as a filler to obtain electrically active biomaterials for MTE applications (6,7).
In addition, the role of different bioactive factors in combination with polymeric scaffolds have been deeply studied for MTE (1). Therapeutic inorganic ions, such as calcium (Ca2+) and zinc (Zn2+), are being studied in applications of tissue regeneration since they induce regeneration avoiding the drawbacks of growth factors (immunogenicity problems, risk of cancer and alterations in cellular homeostasis). In particular, Zn2+, a relevant metallic element in the human body, has been shown to induce proliferation, differentiation, and migration of cells, accelerating in vitro muscle formation (8). It was also proved that extracellular Zn2+ enhances myogenic differentiation by the activation of the Akt signaling pathway (9).
In this study, we hypothesise that bioactive cell environments based on electroactive nanohybrid biomaterials together with Zn2+ ions can synergistically stimulate myogenic differentiation. Conductive PCL/G nanocomposites were prepared with different amounts of G nanoparticles and a non-cytotoxic concentration of extracellular Zn2+ (40 μM) was chosen to analyse its effects in myogenic differentiation with murine myoblasts. The results show that the combination of conductive substrates and extracellular Zn2+ ions (PCL/G/Zn) increases myogenic differentiation in a significant way. However, further studies are needed to explore their full potential in MTE.

Acknowledgments
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keywords: conductive nanocomposites, therapeutic ions, zinc ions, myogenic differentiation, muscle tissue engineering

62825416877

PROBING T CELL MECHANOSENSITIVITY USING ARTIFICIAL ANTIGEN-PRESENTING CELLS

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T cell activation is modulated by signaling molecules on the surface of antigen-presenting cells (APC); however, in recent years, it has become increasingly clear that cellular forces have a crucial role in T cell activation and subsequent effector responses. Therefore, understanding mechanical modulators is critical in advancing current immunotherapy approaches. To address underlying questions, we engineered a biomimetic system to recapitulate the immune synapse, which is the interface of APC-T cell interaction, using polyacrylamide hydrogels with a defined stiffness range comparable to APC stiffness. The hydrogels were functionalized with different ratios of immobilized anti-CD3 (aCD3) and anti-CD28 (aCD28) antibodies. Our results showed that T cell proliferation, cytokine secretion, and intracellular signaling were all reduced at lower gel stiffness. We observed similar results in our cells' models, in which APCs with reduced cell stiffness induced lower T cell activation. To enhance the physiological relevance of the biosystem, we fabricated cell-sized microbeads of varying stiffnesses, then embedded them in 3D collagen matrices. Overall, our biosystem allows decoupling of biophysical and biochemical interactions in T cells activation in a physiologically relevant microenvironment.

keywords: Biomaterials; mechanosensitvity; artificial cells

41883653837

CELL-SELECTIVE ADHESION SHORT PEPTIDES FOR ENHANCING CELL CULTURE ON SCAFFOLD

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Introduction:
Extracellular matrix (ECM) plays a critical role in the control of cell adhesion and growth as in vivo scaffold material, therefore the design of ECM-like or ECM-mimicking scaffold has been one of the powerful concepts to provide a successful culture platform for tissue engineering. However, by their molecular size, it has been a challenge to obtain highly purified ECM molecules. Moreover, it is also extremely costly to obtain ECM molecules to be feasibly used for scaffold material. As a result, such ECM-molecular which can be easily obtained and used for scaffold coating has been limited to several bulk-produced proteins, such as collagens. From the aspect of obtaining pure and highly designable functionalization performance, peptides are advantageous with their synthesis and purification feasibility and their designability for effective surface modification applications. Our group has been investigating short peptides, 3-mer peptides, which can control not only cell adhesion but also “cell-selective adhesion”, to design ECM-mimicking surfaces on cell culture scaffolds [1,2]. The enhancement of cell-selective adhesion is expected to improve the regeneration process on the surface of implantable medical devices because they can provide a material surface to be attractive for “objective cells for treatment”, and be inhibitory for “non-objective cells that disturb treatment”. In this research, we tried to screen novel peptide sequences and combinations to enhance such cell-selective adhesion using peptide array-based direct cell assay [3].

Methods:
On the cellulose membrane, a peptide array was designed using Fmoc peptide synthesis. On the solid-bound peptide array, target cells (endothelial cells, fibroblasts, neural cells, smooth muscle cells) were seeded directly, and their cell adhesion rates were evaluated by a plate reader. We designed various short peptide libraries, containing control RGD cell adhesion peptides, and combined their sequences with various amino acid linker sequences.

Results and conclusions:
By the combinatorial direct screening on peptide array, we found that several short 3-mer peptides can be found to control the cell-selective adhesions to relatively discriminate objective cells and non-objective cells. Moreover, from the data, we found that the short peptide-based cell adhesion effect can be greatly influenced by the linker motifs, the neighboring sequence which provides a physicochemical effect for better or worse cell-peptide interaction. Our results indicate the effective performances of short peptides and their effective surface modification methods to provide a cell-selective effect on cell culture scaffolds. Moreover, our data also suggest that it is important to control the cell-surface interaction mechanism which affects apart from the integrin-mediated adhesion.

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keywords: Peptide array, Peptide functionalization, Cell-selective adhesion

83767204924

ANTIBACTERIAL ALBUMIN-TANNIC ACID COATINGS FOR SCAFFOLD-GUIDED BREAST RECONSTRUCTION

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Introduction
Infection is the major cause of implant failure after breast reconstruction surgery [1]. Medical-grade polycaprolactone (mPCL) scaffolds designed and rooted in evidence-based research offer a promising alternative to overcome the limitations of clinically routinely used silicone implants for breast reconstruction [2-3]. Nevertheless, as with any implant, biodegradable scaffolds are susceptible to bacterial infection, too. Especially as bacteria from the skin can rapidly colonize the mPCL surface during scaffold implantation and form subsequently biofilms. Biofilm-related infections are clinically challenging to treat and can lead to chronic infection and persisting inflammation of the implant host interface [3]. We hypothesize that scaffold guided breast reconstruction combined with an antibacterial implant coating allows to prevent bacterial infection while promoting, at the same time, implant integration and subsequently tissue regeneration.

Methodology
Macroporous scaffolds of a mPCL composite containing 45%(w/w) of sucrose particles with crystals size ranging from 20 to 50 µm, were additively manufactured using a BioScaffolder 3.1 (GeSiM mbH, Germany). The printed scaffolds were immersed in ultrapurified water (AriumR pro UF Ultrapure Water System, Germany) for 15 days in order to leach out the sucrose particles and create microporosity on the surface and within the scaffold struts. Fabricated scaffolds were sterilized by exposure to 70%v/v ethanol followed by evaporation. Scaffolds were then incubated in 1% and 5% human serum albumin (HSA) solutions overnight, at room temperature and under agitation. Resulting coatings of HSA were subsequently stabilized/crosslinked by incubating with 10% or 1%TA. Microporosity of scaffolds, as well its influence on the mechanical properties of clinically relevant large scaffolds was characterized by scanning electron microscopy, microcomputed tomography and uniaxial compression testing. Moreover, 3D in vitro assays were used in order to investigate the stability of the newly developed antibacterial coating and its efficacy against two of the most commonly found bacteria in breast implant-infections, S. aureus and P. aeruginosa.

Results
The physical immobilization of 1% and 5%HSA onto the surface of 3D printed macro- and microporous mPCL scaffolds, resulted in a reduction of S. aureus colonization by 71.7± 13.6% and 54.3± 12.8%, respectively. Notably, when treatment of scaffolds with HSA was followed by tannic acid (TA) crosslinking/stabilization, uniform and stable coatings with improved antibacterial activity were obtained. The HSA/TA-coated scaffolds were shown stable when incubated at physiological conditions in cell culture media for 7 days. Moreover, they were capable of inhibiting the growth of S. aureus and P. aeruginosa, two of the most commonly found bacteria in breast implant infections. Most importantly, 1%HSA/10%TA- and 5%HSA/1%TA-coated scaffolds were able to reduce S. aureus colonization on the mPCL surface, by 99.8± 0.1%

and 98.8± 0.6%, respectively, in comparison to the non-coated control specimens.
Conclusion

This study presents the first set of results for a new biomaterial strategy designed for the prevention of biofilm-related infections on implant surfaces to be used in scaffold-guided breast reconstruction.

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keywords: Bacterial Infection, Antibacterial Coating, Polycaprolactone, Scaffold, 3D Printing

20941826586

POLY(ARGININE) AND HYALURONIC ACID FILM: A MULTIFUNCTIONAL COATING FOR SCAFFOLDS AND INVASIVE MEDICAL DEVICES: THE CASE OF CAVI-T INTRANASAL BALLOON

Cynthia Calligaro (SPARTHA Medical, Strasbourg, France), Sarah Zahouani (Dianosic, Strasbourg, France), Philippe Lavalle (INSERM, Strasbourg, France), Nihal Engin Vrana (SPARTHA Medical, Strasbourg, France)

Introduction: Excessive immune response and development of bacterial infections are two major problems accompanying organ replacement and implant surgeries. Our group aims to develop bioactive coatings to address these issues. Poly(arginine) and hyaluronic acid (PAR/HA) layer-by-layer films are supramolecular thin films (thickness about 1µm) that are easy to build, with promising immunomodulatory and antimicrobial properties 1-4. Here, we investigated the film behavior on CAVI-T intranasal balloon. CAVI-T (Dianosic, France) is a CE-marked medical device which addresses the treatment of intranasal bleeding and which can be used after nasal reconstruction. The device is composed of an inflatable balloon made of polyurethane used for compression of the nasal cavity. A feasibility study was performed with (PAR/HA) film onto polyurethane, looking for film characterization and its antimicrobial activity once deposited onto the balloon, after sterilization, storage and balloon handling, including balloon inflation. As an inflatable medical device CAVI-T is a good testing bed for the modification of mechanically active biomaterial structures.

Methodology: To construct (PAR/HA) multilayer films, polyurethane was alternatively dipped in PAR and HA solutions with intermittent rinsing steps. Coating construction on the substrate was characterized through observation by fluorescence microscopy after film staining with fluorescein isothiocyanate-conjugated PAR. For antimicrobial assays, coated polyurethane was incubated with a Staphylococcus aureus suspension for 24 hours. Planktonic bacteria were quantified by OD600nm measurement in the supernatant. Adherent bacteria were observed at the polyurethane surface after a fluorescent staining of healthy bacteria. Antimicrobial assays were repeated on the device after ethylene oxide sterilization, after accelerated aging and after 10 cycles of inflation/deflation of the balloon.

Results: The best coating construction parameters were defined according to the film homogeneity and thickness. (PAR/HA) films deposited onto polyurethane show a strong bactericidal activity. No bacteria were detected into the supernatant or at the polyurethane surface after 24 hours of contact. After the coating of the device and its sterilization, the antimicrobial activity of the (PAR/HA) remained unchanged. Same results were obtained after an accelerated aging. A final set of experiments was launched after inflation and deflation of the balloon performed into a test tube to mimic the contact with the intranasal cavity during the device introduction. Once again, the antimicrobial activity remained the same.

Conclusion and discussion: These results show the capacity of (PAR/HA) films to ensure antimicrobial activity when coated on the polyurethane balloon of CAVI-T device. (PAR/HA) coating appears to be a simple and powerful system, compatible with an utilization in polyurethane based scaffolds and medical devices. Next step of this study will be the evaluation of the coating for its ability of promoting healing in the specific case of intranasal bleeding. Immunomodulatory and hemostatic properties of the film will be assessed. Thus, the coating design could be tuned to fit polyurethane based scaffolds and devices specific needs.

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Comment: Conflict of Interest:

CC, NEV are full time employees of SPARTHA Medical. SZ is full-time employee of Dianosic SAS. The study was carried out as a pre-clinical feasibility study.

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keywords: Antimicrobial, Biopolymeric biomaterials, Coating, ORL, Supramolecular chemistry

83767225355

INNOVATIVE HYDROGEL TO OVERCOME THE GLIOBLASTOMA THERAPY DEADLOCK

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Introduction
Glioblastoma (GB) is the most frequent and lethal primary brain tumor. GB has currently no cure and the standard care regimens only provide patients a median survival of 12-15 months after diagnosis. Indeed, considering the unfeasibility of performing complete surgical resection and the low efficacy of chemoradiotherapy in eliminating the remaining GB cells, it is not possible to circumvent tumour recurrence. Therefore, there is an urgent need to develop an efficient treatment for GB. This work proposes a hydrogel designed for the direct injection into the resection cavity, allowing starting the treatment immediately after surgery. Being locally administered, it can overcome possible problems related to e.g. systemic side effects and reduced brain penetration. Moreover, the viscoelastic behaviour of the matrix allows the desirable interaction and attachment of the surrounding cancer cells, without the detrimental effects of the tumor microenvironment. Thus, it simultaneously promotes the sustained release of drugs to efficiently damage GB cells while avoiding stimulatory extracellular matrix effects on tumour cells.

Methodology
The hydrogel is based on hyaluronic acid (HA) functionalized with the fibronectin inhibitor peptide Arg-Gly-Asp-Ser (RGDS) and physically crosslinked with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) large unilamellar liposomes (LUVs) encapsulating doxorubicin (DOX). The peptide was synthesized in an automated peptide synthesizer, followed by its characterisation by HPLC-MS. Then, it was used to functionalize HA through carbodiimide chemistry. HA functionalization was confirmed by NMR and ATR-FTIR spectroscopy. LUVs were prepared by the thin-film hydration method followed by extrusion. LUVs' characterisation included the determination of size distribution, surface charge, morphology, phase transition, stability and drug concentration. Hydrogels were characterized in terms of viscoelastic and thermal properties and structure. Drug release profile from hydrogels was evaluated by HPLC. The ability of the matrix metalloproteinase-2 (MMP-2) produced by GB cells to break the peptide-HA binding was also assessed. The human primary GB cell line GBML42 and astrocytes were used to assess the therapeutic value and safety of the developed formulation.

Results
LUVs with DOX presented a homogeneous size of ≈121.7 nm and a slightly negative zeta potential (≈-2.43 mV). Moreover, DOX was encapsulated in relevant concentrations (≈68.2 μM in 1 mM LUVs) considering the DOX IC50 results (≈3.82 μM at 24 h of treatment). The hydrogel presented rheological properties similar to the healthy brain and was able to sustain the release of DOX. In vitro assays demonstrated the efficacy of unmodified HA hydrogels with liposomes encapsulating DOX to damage GB cells. Conversely, RGDS-functionalized HA hydrogels presented cytotoxicity even without DOX incorporation. Indeed, MMP-2 disrupted the peptide-HA bond. Thus, the internalization of free RGDS can lead to GB cells apoptosis. Importantly, RGDS-functionalized HA hydrogels incorporating liposomes with DOX efficiently damaged GB cells

without affecting the metabolism and viability of astrocytes, proving their safety.

Conclusions
RGDS increased the cytotoxicity of the system, proving how it can act synergistically with the incorporated drug for GB treatment. Thus, this work shows the potential of this formulation to be used as a safe and effective local treatment for GB.

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keywords: RGDS functionalized hyaluronic acid hydrogel, liposomes, doxorubicin, cancer cells attachment

41883636764

BUILDING BARRIERS: ENGINEERING A NOVEL IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER

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Introduction: The Blood-Brain Barrier (BBB) is a dynamic interface which regulates the movement of solutes. The physical barrier consists of endothelial cells (ECs) with extrinsic barrier properties induced by interactions with the neurovascular unit (NVU). Neither static nor dynamic in vitro BBB models fully capture in vivo-like conditions, and while coculturing EC monolayers with other NVU cell types has improved barrier properties, the complexity of these culture conditions detracts from their usefulness for high-throughput drug discovery, testing, and disease modelling [1]. The model proposed here utilises material-driven fibronectin (Fn) fibrillogensis by poly(ethyl acrylate), which is biologically compatible with many cell types including ECs, to present a EC monolayer with growth factors (GFs) in synergy with integrin binding sites on Fn [2]. Radically, we hypothesise that an effective in vitro model can be created using GFs and EC monolayer grown on a PEA-Fn coated electrospun membrane scaffold. PLLA electrospun fibres additionally provide a physiological-like scaffold in comparison to commercial polycarbonate or polyethylene-terephthalate semi-porous membranes.

Experimental Methods: Electrospun membranes are produced from poly L-lactic acid (PLLA) 8% solution in hexafluoro-2-propanol and coated with plasma-polymerized PEA (pPEA). Membranes were characterised using scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. hCMEC/D3, were grown on Fn-coated membranes with Fn coating with the addition of GFs at small concentrations (100 ng/ml) on the membrane. Cell/barrier characterisation includes TEER, FITC-dextran permeability and tight junction (TJ) immunofluorescence. Inserts to hold membranes with inbuilt TEER electrodes for continuous resistance measurement in cell culture were designed using AutoDesk Fusion360 and printed on a Prusa 3D printer in PLLA.

Results: hCMEC/D3 cells have low expression of TJs on membranes and their overall TEER values are considered very low. However, we were able to show high level of ZO-1 staining in specific pPEA-Fn-GF conditions, including low permeability to 10/40kDa dextran. Barrier characteristics were further improved by combining different GFs, such as FGF-2 and BDNF, and ECM proteins, such as collagen IV and laminin, and exploration of arranged electrospun fibres in differing geometries. In the investigation of key barrier-inducing GFs, novel GFs which induce higher levels of permeability than VEGF have been discovered.

Conclusion: We demonstrate that this versatile and tuneable design can induce barrier characteristics in immortalised hCMEC/D3 cells. With continued investigation into the changes the cells undergo and optimisation for increased BBB-characteristics, this is primed to be a powerful BBB model. There is room for further optimisation, particularly for the electrospun scaffold and growth factor choice and their combinations, although the model proves promising even at this early stage. This system offers a promising platform, with prospects for the study of

BBB physiology and pathology, as well as for high-throughput BBB drug permeability testing.

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keywords: blood-brain barrier, in vitro models, electrospinning, high-throughput drug testing

62825443626

NOVEL ELASTOMER SURFACE MODIFICATION TECHNIQUE FOR CORNEAL LIMBAL EPITHELIAL STEM CELL INVESTIGATION

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Introduction
Corneal regenerative medicine in recent times has taken a focus on the recapitulation of the limbal epithelial stem cell (LESC) niche. Located peripheral to the central cornea, this pool of stem cells is vital for the preservation of sight throughout adult life. The limbus as an anatomical feature has striking topographical characteristics which are readily observed using clinical ophthalmological equipment. Despite this accessibility, it has remained a challenge to recapitulate its true structure in vitro in a manner which is truly biomimetic both in terms of shape and tissue physical properties. Current methods include structure mimicry techniques, such as lithography [1] and tissue mechanics replication which can involve biological (or biological-like) substrate engineering [2]. In an approach to unify surface shape and mechanical properties, soft polymeric wrinkling [3] was utilized to form a topographical surface for LESC culture and fate control.

Methodology
To produce the substrates, chips of polydimethylsiloxane were exposed to a dual oxidation treatment using strong acid and low temperature oxygen plasma. Subsequent to this, the surfaces were coated with 10% GelMa containing 0.25% LAP through UV crosslinking. Afterwards, substrates were re-hydrated to produce the wrinkled-like surfaces. Human LESC's were isolated from human corneas (NHSBT) using a 2mg/ml Collagenase IV digest of dissected limbal region sections at 37^{0}C overnight. The grown monolayer cells were detached and seeded on the sterilised substrates for up to 7 days culture before fixation. Cell response was characterized using immunofluorescence of key limbal markers: ABCG2, p63, Vimentin, and of key corneal markers: CK3, CK12, Nestin. Imaging was taken using a confocal microscopy. The depth-resolved morphology of the elastomer was examined using Optical Coherence Tomography (OCT).

Results
The substrates have demonstrated regular aligned wrinkles with the dimension (70.86±25.39μm) close to limbal crypt width. OCT images showed consistent crypt depth around 17.14±4.64 μm across the whole substrates. Limbal cells responded to the topographic features well by the clear ordering and alignment with elongation of cells in plane along wrinkle propagation physically. The phenotype change toward the substrate was demonstrated through the changes in marker expression, in particular the marked increase of CK3 and Nestin expression in comparison to monolayer controls in conjunction to a decrease in ABCG2 expression. It is worthy to note that the time in culture on the substrate affected marker expression, stem cell markers were preserved at day 3 but showed transition to corneal commitment at day 7.

Conclusion
In this study, we present a novel fabrication technique to produce biocompatible smart material with physically instructive nature of the topography which is able to replicate the shape of key limbal anatomical features whilst provide mechanobiological signals to exert cell fate control,

driving commitment of LESC's towards epithelial cells.

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keywords: Smart biomaterials, Wrinkling, Topography, Corneal limbal epithelial stem cells

52354563999

THE EFFECT OF AUXETIC METAMATERIAL SCAFFOLDS IN OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Introduction
Severe bone injuries can result in incapacities and thus affect a person's quality of life. Mesenchymal stem cells (MSCs) can be an alternative for bone healing by growing them on scaffolds that provide mechanical signals for differentiation. Such scaffolds can give the appropriate ques to the cells in order to induce their differentiation into mature osteoblasts and later on to be transplanted into the body. Until now lots of attention has been given to create appropriate nano and micro patterns that can work as signal inducers limiting the research in only 2.5 dimensions. On the other hand, our work introduces true, well- defined 3D environments to the research of MSCs differentiation.

Methodology
In our approach, we fabricated hierarchical auxetic mechanical metamaterials and ultra-light ultra-stiff scaffolds via two photon polymerization and used them as scaffolds to investigate the differentiation of MSCs into osteoblasts. Those scaffolds consist of unit cells comparable to the diameter of MSCs which is approximately 50 to 100μm, so only a couple of cells can fit inside thus ensuring the optimal mechanical environment for each cell. In the case of auxetic scaffolds, the unit cells are able to bend without breaking such that the cells can adapt their environment to their needs, whereas the kelvin foam is stiff non elastic scaffold that shows no deformation in response to the forces exceeded by the cells. We investigated the localization of YAP protein, a key protein transcription factor that acts as a mechanotransduction mediator and compared it to common osteogenic markers in both protein and gene levels by using confocal microscopy and qPCR .

Results
Interestingly, YAP protein is translocated to the nucleus even after 21 days of culture and RUNX2 gene shows a 10-fold increase in auxetic scaffold in comparison with the control only after 7 days. Long term cultures up to 28 days shows high mineralization of the extracellular matrix after Alizarin red staining. Moreover, SEM pictures revealed different cell morphology in those different scaffolds because of the different geometries used. Auxetics pushes the cells into more elongated phenotypes whereas kelvin foam in more broad cells bodies.

Conclusion
Auxetic scaffolds are ideal for osteogenic differentiation as they can maintain and promote the osteogenesis efficiently even after 28 days of culture. Our work paves the way for the use of more complicated metamaterials into the tissue engineering field.

Acknowledgement
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keywords: auxetic, metamaterials, osteogenesis, differentiation, multi photon lithography

S15-1
Biologically inspired and Engineered disease models
Room: S1
(28 Jun 2022, 15:30 - 17:00)

Conveners:
Andrew Daly

20967804124

HUMANIZED PLATFORMS BY CONVERGENCE OF BIOMATERIALS, CELLS AND MICROTECHNOLOGIES

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The functionality of living organs is intimately linked to their complex architecture, from the physicochemical properties of extracellular microenvironment, to tissue-level scale, where multiple cell populations interact in a precisely orchestrated spatial distribution. Recent technological advancements in the field of microfluidics, biomaterials and tissue engineering have enabled new approaches to design and fabricate in vitro models of various pathophysiological conditions, one step further to organ level mimetics. Compartments in microchannels separated by membranes, flexible thin films with embedded electrodes and co-cultures represent the basics in the design of some pathology models that have been developed so far. As for the tissue construct, elicitation of cell types and the microenvironment are crucial. Both primary cells and human induced pluripotent stem cells (iPSC) are utilized in such platforms. In the majority of the cases, biopsies from patients form the basis, whereas genetic tools such as CRISPR-Cas9 genome editing technologies is utilized for creating mutations in iPSCs to mimic a variety of genetic disorders. On the other hand, the recapitulation of the unique microenvironment requires gaining insights to the components of the extracellular matrix, imposing the significance of proteomics analyses. Decellularization of respected tissues, their synthetic counterparts or hybrid hydrogels are investigated to recreate such microenvironments. Last but not the least, the cell culture media representing the blood flow is another issue to consider. Overall, humanized platforms for disease modeling have great prospects for adjusting the variables related to the pathophysiology, measurement, and long-term observation of disease progression. Although the field is still in its infancy, developing disease model-on-a-chip platforms will enhance our understanding of the mechanism and progression of diseases, as well as improve drug developments.

Keywords: engineered disease models; humanized platforms; induced pluripotent stem cells; extra cellular matrix; tissue decellularization

Acknowledgement: The financial support provided by TUBITAK through grant no. 119M578 is highly appreciated.

keywords: engineered disease models; humanized platforms; induced pluripotent stem cells; extra cellular matrix; tissue decellularization

62825406088

TUNING MACROPHAGE POLARIZATION TO MODEL MYOCARDIAL INFARCTION IN THE GENERATION OF FUNCTIONAL CARDIAC ORGANOIDS

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INTRODUCTION: Myocardial infarction (MI) is an ischemic and inflammatory event majorly orchestrated by macrophages from infiltrating monocytes. These macrophages play a critical role in deciding the fate of the heart post-MI. However, there is no cardiac disease model in existence that incorporates an immune response. Hence, the aim of this project is to develop a humanized model of MI, using induced pluripotent stem cell (iPSC) derived cardiomyocytes together with inflammatory cytokine stimulation, to model the disease environment.

PURPOSE: Despite the advances in developing effective engineered heart tissue (EHT) models for MI that can recapitulate intricacies of the native myocardium, such as contractile properties and ability to respond to different chemical stimuli, there is still a need to make these models physiologically relevant. We hypothesize that the addition of macrophage-derived inflammatory cytokines can aid in making EHT models of MI more humanized.

METHODS: The first objective of this project is to obtain conditioned media obtained from immune cells for stimulating cardiomyocytes. In order to achieve this, iPSCs were differentiated to obtain macrophages (iMacs). Their expression of general macrophage (CD14, CD11b) and resident macrophage (CX3CR1, CCR2 and HLADR) markers were assessed, in addition to their phagocytic and polarization potentials. Next, iPSC-derived cardiomyocytes were obtained (iCMs) and the experssions of general cardiomyocyte (cTnT, cx43) and maturation (sarcomeric actinin) markers were assessed. Finally, polarizsation of iMacs were analyzed within a collagen/matrigel hydrogel system.

RESULTS: iMacs were found to be 93.6% CD14highCD11bhigh. Compared to blood-derived macrophages, CCR2 was downregulated and CX3CR1 and HLADR were upregulated in iMacs showing a resident macrophage phenotype. Additionally, iMacs also showed phagocytic potential (39.9%) and ability to be polarized to pro-inflammatory (on stimulation with LPS and IFN-gamma) and anti-inflammatory (on stimulation with IL4) states. iCMs were found to be positive for cTnT, cx43 and sarcomeric actinin. Additionally, they were found to be positive for MLC2a, denoting an atrial cardiomyocyte phenotype.
iMacs were found to not polarize within the collagen/matrigel hydrogel system, which will be used as the final model to develop the EHT.

CONCLUSIONS: Macrophages with a higher resident phenotype and have been generated. In the future, conditioned media from these iMacs stimulated with pro-inflammatory factors will be used to treat cardiomyocytes, to understand its effects on cardiomyocyte function.

keywords: induced pluripotent stem cells, myocardial infarction, disease model, immunoengineering, macrophages

83767214567

RECONSTRUCTION OF FUNCTIONAL GRADIENTS USING MELT ELECTROWRITING

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Introduction
Melt Electrowriting (MEW) is a biofabrication approach that combines principles of electrospinning and 3D printing. It allows obtaining the porous scaffolds with well-defined fibers in the range of a few micrometers with unprecedented precision. In our group, we use this approach to reconstruct the structure of different native tissues and model their functions in vitro.
This project aims to develop a functional reconstruction of human trabecular meshwork (HTM). HTM is a mesh located in the eye, responsible for the drainage of liquid for the anterior chamber and, therefore, maintaining the proper pressure in the eye. When dysfunctional, it often leads to glaucoma development.

Methodology
Different designs of porous scaffolds, closely mimicking the structure of native HTM, were designed and printed using MEW. Polycaprolactone was used for printing, and the scaffolds were coated with poly-L-lysine for cell culture studies. Primary HTM cells were seeded, and their performance was characterized in 21-days culture. Mechanical properties of the scaffolds were analyzed in tensile testing and tuned to obtain values matching healthy and glaucomatous eyes. To analyze the functionality of the proposed models, the permeability test was performed on cell-seeded scaffolds at the physiological liquid pressure.

Results
The scaffolds with the architectures resembling three distinctive zones of native HTM were successfully obtained. HTM cells cultured on the prints revealed high viability and expression of cell-specific proteins. Tensile test results revealed that mechanical properties close to healthy or glaucomatous tissue could be obtained by varying the scaffolds' design. Cell-seeded scaffolds showed permeability values relevant for native tissue.

Conclusions
The MEW approach allowed to obtain structures closely mimicking native HTM regarding the design, mechanical properties, and function (i.e., permeability). We envision that these scaffolds will find applications as in vitro testing platforms for glaucoma drugs and, after further optimization, as implants for patients that require removal of dysfunctional HTM. This is an especially impactful finding as adequate HTM in vitro models, and biomimetic HTM implants are currently missing.

keywords: Human Trabecular Meshwork, Melt Electrowriting, gradient scaffolds, tissue engineering, in vitro models

83767220867

TISSUE ENGINEERING A HUMANIZED RAT MODEL FOR OSTEOSARCOMA RESEARCH

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Introduction:
Osteosarcoma (OS) is the most frequent malignant bone tumour with a survival rate of less than 25% in metastatic disease. Treatment is a combination of surgical resection and systemic chemotherapy with doxorubicin (DOX). However, this can cause serious long-term adverse effects. To overcome this limitation, we have developed an advanced rat OS model that converges tissue engineering and regenerative medicine principles to improve treatment outcomes for OS patients.

Methodology:
A humanized bone microenvironment was created by implanting an orthotopic tissue engineered bone construct (ohTEBC) fabricated from melt electrowritten medical grade polycaprolactone coated with calcium phosphate (mPCL-CaP) together with fibrin glue (Baxter) and 45 µg rhBMP-2 (Medtronic) around the femur of an immunocompromised rat. A primary OS tumour was then created by injecting human SaOS-2-luc human OS cells into the humanized bone niche. Bone volume was measured with in vivo CT (Molecubes µPET-CT) and tumour formation and metastasis was monitored using bioluminescent imaging (BLI) (IVIS Spectrum, Perkin Elmer). The humanized bone niche and primary OS tumour was characterised by ex vivo histology and immunohistochemistry.

Results:
A humanized bone microenvironment formed within 6 weeks of implantation, with µCT confirming increased bone volume around the femur. Following SaOS-2-luc injection, BLI confirmed primary OS tumour growth and development of lung metastases over a 14-week period. µCT revealed pathological increase in bone volume within the OS tumour. In future studies, the humanized rat OS model will be used to investigate the efficacy of scaffold-mediated local DOX delivery following surgical resection of the primary OS tumour, in comparison to systemic DOX chemotherapy delivery. The model will also be used to study the regeneration of post-DOX-treated critical-sized segmental bone defects using scaffold-guided tissue engineering and regenerative medicine approaches.

Conclusion:
Here, we have created an orthotopic OS tumour model that recapitulates the hallmarks of human disease within an immunocompromised rat. This model will allow to study complex surgical interventions and regenerative medicine techniques never before possible in previous model systems. The outcomes of this study will improve the chemotherapeutic and limb sparing surgery options for those affected by OS.

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keywords: osteosarcoma, tissue-engineered bone, surgical resection, local chemotherapy, scaffold-guided bone regeneration

83767225105

ELECTROSPUN PATCH DELIVERY OF ANTI-TNFA F(AB) ANTIBODY FRAGMENT FOR THE TREATMENT OF ORAL MUCOSAL INFLAMMATORY DISEASES

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Introduction: Chronic ulcerative oral mucosal inflammatory diseases, including oral lichen planus and recurrent aphthous stomatitis, are painful and highly prevalent, yet lack effective clinical management. These conditions are usually treated with either topically applied or systemically-delivered corticosteroids depending on severity with varying degrees of success. In recent years systemic biologic therapies, including monoclonal antibodies that block the activity of proinflammatory cytokines, have increasingly been used to treat similar immune-mediated inflammatory conditions, such as rheumatoid arthritis and psoriasis. The ability to deliver these therapies locally to the oral epithelium could radically alter treatment options for oromucosal inflammatory diseases, where cytokines, in particular tumour-necrosis factor-a, are a major driver of pathogenesis.
We previously developed a dual-layer electrospun mucoadhesive patch with high patient acceptability and a long in vivo residence time (Colley, HE et al., Biomaterials. 178:134-146 (2018)). We have incorporated small molecules drugs such as corticosteroids and anaesthetics into these patches and have demonstrated their release and activity (Clitherow KH, et al., Mol Pharm 16:3948-56 (2019)). Recently, we showed that small proteins and peptides can also be incorporated whilst retaining their biological activity (Edmans JG. et al., Mat Sci Eng 112:110917 (2020)). Here, we investigate the inclusion and delivery of therapeutic anti-TNF-α F(ab) antibody fragments for the treatment of oral inflammatory disease.

Methodology and results: Biotinylated F(ab) fragments was incorporated into an electrospun mucoadhesive membrane and were found to be retained within the fibres in aggregates when visualised by confocal microscopy. These F(ab) were rapidly eluted from the patch without loss of antigen binding activity (97 ± 5% released within 3h). Neutralising anti-TNF-α F(ab) fragments were generated from whole IgG by papain cleavage, as confirmed by SDS-PAGE, then incorporated into patches. Antibody-containing patches were found to have TNF-α neutralising activity, as shown by the suppression of TNF-α-mediated CXCL8 release from oral keratinocyte grown as monolayer cultures. Patches applied to lipopolysaccharide stimulated immune-competent oral mucosal ulcer equivalents that contained primary macrophages, led to a statistically significant reduction in the levels of biologically active TNF-α, suggesting successful delivery of a therapeutically relevant dose. Moreover, inhibition of TNF-α by patch-released anti-TNF-α F(ab) fragments also resulted in a significant decrease in the levels of T-cell chemokines produced, indicating that patch-delivered neutralising antibody therapy impacts on downstream immunological events such as leukocyte recruitment that will further prevent pathogenesis.

Conclusion: Electrospun oromucosal patches can deliver active biologics such as therapeutic antibodies topically to diseased oral mucosal sites in vitro. These patches have the potential to change the way these debilitating oral diseases are treated in the future.

keywords: Electrospinning, biologics, oral medicine, drug delivery

52354539126

DOX-LOADED MPEG NANOPARTICLES AS A PROMISING TREATMENT IN A HUMANIZED MOUSE MODEL FOR BREAST CANCER BONE METASTASIS

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Breast cancer (BCa) is the most common cancer amongst women worldwide and the leading cause of cancer related death. The triple negative BCa (TNBCa) subtype is associated with a particularly aggressive clinical behavior including an early peak of fatal distant metastasis, predominantly to bone. Routine systemic chemotherapeutic treatment with doxorubicin (DOX) is limited due to its severe side effects, especially cardiotoxicity. Novel (targeted) nanotherapies are a promising breakthrough to enhance treatment efficacy and specificity of known chemotherapeutics while at the same time decreasing their systemic toxicity. Therefore, we studied the therapeutic effect of non-targeted and targeted DOX-containing nanoparticles as a novel treatment approach against TNBCa bone metastases using a humanized tissue-engineered mouse model.

A humanized bone microenvironment was created in NSG mice by subcutaneous implantation of humanized tissue-engineered bone constructs (hTEBCs) consisting of tubular biodegradable medical grade polycaprolactone scaffolds seeded with human osteoblasts and an inner vascular bone marrow niche. After 13 weeks of in vivo bone formation, TNBCa primary tumor was

induced by injecting MDA-MB-231BO-Luc cells into the mammary fat pad and the tumor was allowed to grow and metastasize to humanized bone. Subsequently, treatment with DOX-loaded hyper branched methoxy polyethylene glycol (mPEG) (HBP) nanoparticles was performed. The DOX-HBP nanoparticles were either non-targeted or targeted with a Thomsen-Friedenreich (TF)-mPEG bispecific antibody (BsAb) and administered once per week over a duration of 3 weeks.

Implantation of the hTEBCs resulted in the formation of a chimeric bone organ in vivo containing human-derived extracellular matrix, bone marrow and showing evidence of ongoing complex bone formation through endochondral ossification. HBP nanoparticles predominantly accumulated at the primary tumor and hTEBCs. The non-targeted HBP-DOX nanoparticles were able to slow primary tumor growth and reduce metastasis compared to the targeted HBP-DOX nanoparticles and non-DOX containing control groups. Additionally, the non-targeted nanoparticles reduced systemic toxicity effects (cardiotoxicity, hepatotoxicity, hematological toxicity) and prolonged survival compared to free DOX treatment. Further targeting with the BsAb did not improve treatment outcome, most likely because of enhanced clearance (accumulation in liver and spleen). However, the targeted HBP-DOX nanoparticles lead to increased lung metastases and tended to increase metastasis to the liver and hTEBCs compared to the saline control.

In conclusion, this study is an exciting example of complex pre-clinical disease modelling including a humanized bone niche in the mouse. Furthermore, it highlights the great potential of nanomedicines in cancer therapy, but also demonstrates how changed nanoparticle properties can alter their treatment efficacy in vivo.

keywords: humanized animal model, bone metastases, triple-negative breast cancer, nanomedicine, chemotherapy

52354528084

A 3D IN VITRO MODELS OF IMPAIRED OSTEOCYTES ACTIVITY UNDER EXPSOURE TO INDOXYL SULFATE

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Introduction
Patients with chronic kidney disease (CKD) experience multiple comorbidities, among which mineral/bone disorders (MBD) contribute to high mortality due to increased facture risk [1]. CKD affects the quality of bones which become weaker and easily break. Recently, endogenous metabolites, such indoxyl sulfate (IS), were reported to have an active role in the development of uremic bone as they accumulate in blood due to poor kidney function [2]. However, the effects of these metabolites on the bone matrix is not well understood, due to lack of appropriate models. For this, we established an in vitro 3D model of human bone to investigate the activity of osteocytes, master regulators of bone remodeling, under exposure to IS. We investigate the osteocyte phenotypic signature (gene and secretion of remodeling molecules) accompanied by analysis of the quality and quantity of the deposited bone matrix.

Materials and Methods
We encapsulated human adipose-derived stem cells (hASCs) into fibrin hydrogels and differentiated them into osteoblasts- and osteocytes-like cells. The osteogenic differentiation was performed either under standard conditions or under treatment with IS. To assess the effect of IS treatment on the newly formed bone-like matrix, we examined the differential expression of the major osteoblastic (Opg, Col1a1, Opn, Osteonectin, Tnfsf11) and osteocytic genes (Fgf23, Col1a1, Mepe, Sclerostin, Opg, Osteonectin), and evaluated the secretion of soluble key regulators of bone remodeling (OPG, FGF23, SOST). The composition and mineralization of the bone matrix was also analyzed by staining for calcium (Alizarin Red) and collagen (Sirius Red and immunostainings), accompanied by mechanical testing of the bone-like construct.

Results
HASCs successfully differentiated into osteoblasts and osteocytes-like cells in standard osteogenic differentiation conditions, as evidenced by a highly mineralized and collagen enriched matrix. IS treatment was shown to significantly downregulate the expression of bone remodeling genes. Osteocytes-secreted bone remodeling mediators were also significantly lower ($p<0.05$) when compared to healthy cultures. Even more, the quality and the quantity of the deposited bone were impaired. The amount of collagen, as well as the degree of mineralization were reduced by 43.9%, $p<0.001$ and by 29.7%, $p<0.05$, respectively. Via X-ray imaging, we could identify a poor calcium-containing matrix while under exposure to IS. These changes were reflected in the decrease ($p<0.05$) of the mechanical properties of the uremic bone-like construct.

Conclusion
Our results suggest that IS impairs the osteogenic differentiation of hASCs and induces alterations of the bone extracellular matrix, in terms of collagen deposition, amount of inorganic matter and mechanical properties. Furthermore, exposure to IS during the transition of osteoblasts to osteocytes affects the acquisition of key regulatory features of bone remodeling.

Acknowledgments
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keywords: uremic bone, osteocytes, indoxyl sulfate, in vitro model

31412705605

GLYCOTRIPEPTIDES SHOWCASE THE EFFECT OF GLYCOSYLATION ON PROTEIN AGGREGATION

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Introduction
Folding is a crucial process that modulates the function of proteins:[1] non-covalent intramolecular interactions between amino acids give rise to a defined three-dimensional structure with minimal free energy known as protein native state. This is an error prompt process that often results in misfolding and the formation of off-pathway aggregates associated with pathological conditions. There are different cellular mechanisms to control this process, e.g., post-translational modifications that change the free energy of the protein. An example is glycosylation that affects the protein folding and aggregation.[2] The thermodynamics and kinetics of these processes are still poorly understood and often rely only on in silico models. Herein, we show that O-glycotripeptides are extremely useful reductionist models to study the involvement of glycosylation in these processes at molecular and microscopic levels.[3]

Methodology
Minimalistic glycoproteins were designed using glycosylated serine (S) or threonine (T) flanked by phenylalanine (F). The glycosylated S and T are characteristic structural components of O-glycoproteins, while the aromatic F was introduced to augment the aggregation propensity of the glycopeptides. The aggregation of these glycotripeptides was compared to their respective non-glycosylated analogues using in silico all-atom molecular dynamics simulations and in vitro by circular dichroism (CD) and X-ray diffraction (XRD). The morphology of the generated aggregates was visualized by scanning (SEM) and transmission (TEM) electron microscopies and their mechanical properties were measured by atomic force microscopy (AFM).

Results
We were able to assess the distinct contributions of F, S or T and glucose to the glycopeptides' stereochemistry and aggregation. Although S and T differ only by a methyl group, this subtle variation affects the inter- and intramolecular CH-pi interactions between F and S or T: F/S << F/T. S to T substitution also induced alterations in the morphology of the generated supramolecular aggregates as shown for the non-glycosylated peptides. O-glycosylation introduced changes in the pi-interactome by establishing additional CH-pi interactions, i.e., Glc/F. The aggregates of the

glycopeptides have reduced stiffness and increased thermal stability when compared to their non-glycosylated counterparts. These changes were more prominent for the S analogues when compared with the T ones.

Conclusions
We demonstrate that simple glycotripeptides are a useful model for revealing the mechanism(s) of the aggregation processes at the molecular level. The generated assemblies can be also used as functional biomaterials acting as biomimetics of glycoproteins.

Acknowledgements
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keywords: Protein Aggregation. Glycosylation. Glycotripeptides. Glycoproteins.

S15-2
Biologically inspired and Engineered disease models
Room: S1
(29 Jun 2022, 15:30 - 17:00)

Conveners:
Y. Shrike Zhang

73296323247

INVESTIGATING THE EFFECT OF APOLIPOPROTEIN E4 ON PERICYTE CONTRACTION

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Introduction:
Pericytes reside outside of capillary blood vessels. In the brain, pericytes, brain microvascular endothelial cells, and astrocytes form the neurovascular unit (NVU). A normally functioning NVU regulates cerebral blood flow and the permeability of the blood-brain-barrier (BBB). Breakdown of the NVU and dysfunction of the BBB are increasingly recognised early biomarkers of dementia, including Alzheimer's disease. An isoform of Apolipoprotein E (APOE), namely APOE4, which is the strongest genetic risk factor for Alzheimer's disease1, has a particular importance in BBB dysfunction compared to the E3 variant. Pericyte dysfunction has received increasing recognition in Alzheimer's disease where pericytes contract capillaries and thus reduce cerebral blood flow, in a downstream response to amyloid-β2. However, pericytes in health and disease remain poorly understood, partially due to a lack of adequate models that can recapitulate the complexity of the multi-cellular NVU. The aim of this study is to investigate the effect of APOE4, compared to APOE3, on the contraction of human pericytes in vitro.

Methodology:
iPSCs from a donor diagnosed with Alzheimer's disease carrying the APOE e4/e4 allele (UKBi011-A, EBiSC) as well as the isogenic control with the APOE e3/e3 isotype (UKBi011-A-3, EBiSC) were differentiated into pericytes3. Success of differentiation was confirmed by immunohistochemistry of pericyte markers. The labelled calcium indicator Fluo4 was used to measure increase in fluorescence, thus increase in calcium release, within the iPSC-derived pericytes.

Results:
APOE3- and APOE4-pericytes stained positively for NG2, PDGFR-β, calponin, and SM22. Genomic sequencing confirmed the isoforms of APOE3 and APOE4. Fluo4 staining was used to observe calcium release, which was triggered by two known vasoconstrictors Endothelin-1 and U46619, the latter one being a thromboxane A2 receptor agonist. Calcium firing was observed for a period of at least 10 minutes. Change in cell area and calcium increase were quantified using an automated image analysis pipeline in CellProfiler.

Conclusion:
These results suggest that functional pericyte-like cells were obtained and that their calcium release could be triggered. We will build on these findings to develop a robust model to study the effect of APOE4 on pericyte function and dysfunction in health and Alzheimer's disease.

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keywords: Pericytes, iPSC, Alzheimer's disease

20941816389

INACTIVATED SARS-COV-2 VIRAL PARTICLES PROMOTE CILIATION IN TISSUE-ENGINEERED 3D AIRWAY TRI-CULTURES

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Introduction
Despite the availability of safe and effective vaccines against COVID-19, new variants of concern (VOC) such as Delta and Omicron are emerging. In these surges, governments and institutions around the world urgently need ways to rapidly respond to outbreaks of new VOCs or other infectious respiratory diseases with pandemic potential.

In this context, tissue-engineered models serve as valuable tools for immediate preclinical and translational research regarding therapeutics and prophylactics. Models faithfully mimicking human respiratory mucosa and submucosa could support research and development regarding COVID-19 and other diseases caused by pathogens entering the body by the respiratory route.

Methodology
Human bronchial fibroblasts (HBFs) isolated from cryo-biopsies were combined with human umbilical vein endothelial cells (HUVECs) inside 3D fibrin hydrogels. Primary human bronchial epithelial cells (HBEs) were seeded on top of the gels, which were then grown on transwell inserts for one week as submerged culture followed by 4 weeks of air-liquid interface culture. The models were fixed and CD31/PECAM-1 and the SARS-CoV-2 entry receptor hACE2 were analyzed by 2-Photon Laser Scanning Microscopy (2P-LSM) and immunohistochemistry, respectively. Furthermore, cultures were examined by Scanning Electron Microscopy (SEM).

SARS-CoV-2 viruses were cultured in Calu-3 cells, isolated, and added from either the apical side, the basal, or both. Cultures were fixed 8 days post-treatment and immunostained for the viral

Nucleocapsid (N) protein to visualize the infection. 2D monocultures of HBFs and HUVECs were also infected and analyzed for spike (S) and N protein expression by a newly developed in-cell-ELISA1.

Additionally, a clinical isolate of the VOC Delta was cultured in Calu-3 cells to prepare virus particles that were inactivated by ultraviolet light (UV) irradiation ("UV-Delta"). UV-Delta was added to the aforementioned airway 3D models, which were incubated for 4, 24, or 48 hours, fixed, and examined by histological periodic acid Schiff´s (PAS) reaction and SEM.

Results
Similar to native tissues, histology of the airway tri-cultures showed high expression of the SARS-CoV-2 receptor hACE2 mainly in the epithelium layer. The 2P-LSM 3D stacks of the CD31/PECAM-1 staining showed the HUVECs arranged in a network of capillary-like structures, while the SEM confirmed the presence of a fully developed ciliated epithelium on the apical side of the model.

While the icELISA detection in 2D monocultures verified that both HUVECs and HBF are in principle susceptible to SARS-CoV-2 infection, the N protein immunostaining of tri-cultures revealed that complex models are only permissive to SARS-CoV-2 when exposed to the virus from the epithelium side. The PAS reaction showed a weakening and thinning of the epithelial barrier when exposed to UV-Delta, while the SEM analysis revealed an increase in ciliation of the epithelium after 24 and 48 hours.

Conclusion
Our airway tri-cultures represent a complex model for research regarding the pathogenesis of SARS-CoV-2 and therapeutics against COVID-19. The treatment of the tri-cultures with inactivated viral particles from the VOC Delta induces an increase in ciliation, a counterintuitive effect that differs from the findings with live SARS-CoV-2 and other coronaviruses.

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keywords: SARS-CoV-2, COVID-19, Disease model, Airway, Epithelium

20941813149

A TISSUE ENGINEERING MODEL OF CRANIOSYNOSTOSIS TO IDENTIFY NEW THERAPEUTIC TARGETS THAT ACCELERATE BONE HEALING IN ADULTS

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Introduction: Craniosynostosis (CS) is a bone developmental condition that affects 1 in 2100 children worldwide, characterised by premature ossification of the cranial sutures. Particularly, non-syndromic-CS (NS-CS) has been associated to microenvironmental causes. However, little is known about the signalling pathways that govern this skull suture premature ossification. Thus, we hypothesize that by investigating the role of microenvironmental cues in NS-CS, we can identify novel ossification therapeutic targets that could be utilised to develop novel biomaterials-based therapeutic treatments for bone fracture healing in adults.

Methodology: Cells were isolated from patent (unfused) sutures, fused sutures and calvarial bone of children (5-28 months) diagnosed with NS-CS. Tissues were collected during cranial vault remodelling –standard CS surgical procedure- at CHI at Temple Street after parental consent and ethical approval were obtained[1]. To evaluate their osteogenic potential, alkaline phosphatase (ALP) activity and extracellular matrix mineralization of cells cultured for 7-21 days in growth (GM) and osteogenic medium (OM) were quantified. Subsequently, to understand how variations in the substrate stiffness affect premature ossification, cells were cultured on soft (10 kPa) and stiff (300 kPa) collagen-coated polyacrylamide substrates[1]. Then, their osteogenic potential and morphological responses were evaluated. The differences in the mechanoresponse of these cells were further investigated with a 96 gene PCR array to identify potential therapeutic targets[1].

Results: Cells from patent and fused sutures expressed similar ALP activity and extracellular matrix mineralisation at the different evaluated time-points, when cultured with GM. Interestingly, when cultured with OM, cells from fused sutures expressed higher mineralisation levels and ALP activity. Thus, suggesting that cells from fused sutures have a stronger osteogenic response than cells from patent sutures when biochemically stimulated. Furthermore, when cultured with GM on soft and stiff substrates, cells from both patent and fused sutures exhibited morphological changes and increase in their spreading area, in a stiffness-increasing manner. Particularly, cells from fused sutures showed a bigger and rounded shape, resembling osteoblasts while cells from patent sutures were elongated, resembling mesenchymal stem cells. Finally, when combining variations in the substrate stiffness and OM, a stiffness-dependent upregulation of genes mediating bone development (TSHZ2, IGF1), activation of inflammation (IL1β), involved in the breakdown of extracellular matrix (MMP9) and controlling osteogenic differentiation (WIF1, BMP6, NOX1), was observed in cells from fused sutures. These findings suggest that the increased osteogenic potential of cells from fused sutures might be associated

to the activation of the BMP6, IGF1 and/or MAPK-associated non-canonical WNT pathways.

Conclusions: Our results further suggest that NS-CS may be linked to an abnormal mechanical environment. Understanding the changes in the regulation of genes associated with the premature suture ossification in CS opens up avenues to not only understand better this developmental condition but also will help us to design novel therapeutic strategies to accelerate non-union bone fracture healing in adults.

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keywords: Bone Healing, Craniosynostosis, TE Models

20941829709

TOWARDS THE DEVELOPMENT OF MULTIAXIAL LOADING BIOREACTOR FOR INTERVERTEBRAL DISC STUDIES: VALIDATION OF AN EX VIVO ORGAN MODEL AND CUSTOMIZED SAMPLE HOLDER

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Introduction
The intervertebral disc (IVD) distributes multiaxial loads applied to the spine, namely axial compression, tension, lateral bending, and torsion. The effect of mechanical loading on IVD health and degeneration is commonly investigated in bioreactors used for ex vivo culture of IVD organ models. Currently available bioreactors have mainly integrated one or two degrees of freedom (DOF), while thus far developed multiaxial simulators with 6 DOF lack control of biological conditions. A new generation of bioreactors will integrate both six DOF loading and sterile culture of IVD organ models. Such a multiaxial system requires the implementation of the holding mechanism that must enable proper transmission of the loads from the bioreactor onto the specimen and sufficient nutrition through the cartilaginous endplate. We developed and validated a sample holder and new ex vivo IVD organ model according to the requirements for multiaxial bioreactor culture.

Methodology
A customized, circular IVD holder with a central cross pattern was designed. An ex vivo bovine caudal IVD organ model was adjusted to maintain more vertebral bone than the standard model (5-6 mm instead of 0.5 mm) to machine the cross counterpart and a central hole for nutrient access. The new and standard models were compared for long-term maintenance in a bioreactor under physiological conditions by alternating cyclic compressive uniaxial loading (0.02-0.2 MPa, 0.2 Hz, 2h/day) and overnight free swelling recovery. The disc height changes were measured daily, and cell viability was assessed with histology after 1, 2 and 3 weeks of culture (n= 2 for each time point) in comparison to day 0 samples (n= 3). The interface of the new IVD model and sample holder was enhanced with tightening of side screws onto the bone, or a combination of side screws and top screws, or side screws and adhesive, and was tested for failure point in compression, tension, torsion, and lateral bending (n=3 for each test).

Results
The new model retained a high level of cell viability during three weeks of in vitro culture (standard versus new model after 3 weeks: outer annulus fibrosus 82% and 84%, inner annulus fibrosus 69% and 64%, nucleus pulposus 75% and 73%). In both models, the decrease in IVD height after loading was in the range of typical physiological conditions (≤ 10%). When differently directed motions were applied, the holder-IVD interface with side screws transmitted compression and torsion above reference values (average obtained values were 320.37 N and 1.64 Nm, respectively), while the combination of side and top screws improved the resistance to tension and bending compared to the targeted values (average obtained values were 431.86 N and 0.79 Nm, respectively).

Conclusion
We have developed a mechanically reliable holding system for application in a new generation of multiaxial bioreactors and demonstrated that the new ex vivo IVD organ model can be maintained in long-term culture. Additional studies are envisaged to validate the system in the new bioreactor. Such a unique bioreactor will enable overcoming the gap between preclinical in vitro cultures, animal models, and clinical trials.

keywords: bioreactor, intervertebral disc, multiaxial loading, organ model, sample holder

62825463608

CULTURE OF CANCER SPHEROIDS AND EVALUATION OF ANTI-CANCER DRUGS IN 3D-PRINTED MINIATURIZED CONTINUOUS STIRRED TANK REACTORS (MCSTRS)

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Cancer continues to be a leading cause of mortality in modern societies; therefore, improved and more reliable in vitro cancer models are needed to expedite fundamental research and anti-cancer drug development. Here, we describe the use of a miniaturized continuous stirred tank reactor (mCSTR) to first fabricate and mature cancer spheroids (i.e, derived from MCF7 cells, DU145 cells, and a mix of MCF7 cells and fibroblasts), and then to conduct anti-cancer drug assays under continuous perfusion. This 3 mL mCSTR features an off-center agitation system that enables homogeneous chaotic laminar mixing at low speeds to support cell aggregation. We incubated cell suspensions for 3 days in ultra-low-adherence (ULA) plates to allow formation of discoid cell aggregates (~600 µm in diameter). These cell aggregates were then transferred into mCSTRs and continuously fed with culture medium. We characterized the spheroid morphology and the expression of relevant tumor biomarkers at different maturation times for up to 4 weeks. The spheroids progressively increased in size during the first 5 to 6 days of culture to reach a steady diameter between 600 and 800 µm. In proof-of-principle experiments, we demonstrated the use of this mCSTR in anti-cancer drug testing. Three drugs commonly used in breast cancer treatment (doxorubicin, docetaxel, and paclitaxel) were probed at different concentrations in MCF7 derived spheroids. In these experiments, we evaluated cell viability, glucose consumption, spheroid morphology, lactate dehydrogenase activity, and the expression of genes associated with drug resistance (ABCB1 and ABCC1) and anti-apoptosis (Bcl2). We envision the use of this agitated system as a tumor-on-a-chip platform to expedite efficacy and safety testing of novel anti-cancer drugs and possibly in personalized medicine applications.

keywords: cancer, spheroids, stirred tank, continuous, 3D-printing

73296342489

COLLAGEN-BASED 3D CO-CULTURE MODEL TO INVESTIGATE THE MULTIPLE MYELOMA MICROENVIRONMENT IN BONE MARROW

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Introduction
Plasma cell malignancy - multiple myeloma (MM), occurs primarily in the bone marrow (BM) and appears to be strongly regulated by interactions with BM mesenchymal stromal cells (MSCs). In addition, collagen is an important constituent of the BM environment and provides not only structural, but functional support to many of its cells. Improved understanding of MM pathology requires a development of 3D models that can recapitulate the BM-supported MM survival and intercellular communication. Therefore, we aimed to establish a collagen-based 3D co-culture model of MM cells and MSCs.

Methodology
MSCs were obtained from BM of patients undergoing total hip arthroplasty. For 3D co-culture, MSCs and semi-adherent MM cells (MM1.S) were grown in hydrogels generated using rat-tail collagen type I. For mono-cultures, 3 x 105 cells were cultured per 100 µl of collagen, whereas in co-culture, the cells are seeded in an initial one to one ratio, giving a total of 3 x 105 cells (2D controls were performed in parallel). Viability and metabolic activity of encapsulated cells were examined performing an MTT assay and measuring ATP concentration at 1, 3 and 7 days of culture. Gene expression was assessed by qPCR and proliferation evaluated using flow cytometry. Immunohistochemistry was performed to identify functional marker changes. Finally, clonogenic capacity of both cell types was determined after retrieval from collagen gels.

Results
Both MM1.S cells and MSCs were successfully encapsulated and cultured in collagen gels. Upon day 7, the portion of MM1.S cells exceeded the percentage of MSCs in the 3D co-culture system, where MM1.S cells accounted for about two-thirds of total retrieved cells. The viability of the cells did not seem to be affected by a collagen-based environment. However, the proliferation of MM1.S cells appeared changed, with a decreased Ki-67high fraction of MM1.S cells growing in 3D culture, suggesting a attenuated proliferation potential, regardless of the presence of MSCs. Reduced metabolic activity and ATP production were observed in 3D cultured MM1.S cells and MSCs in comparison to standard 2D culture. In addition, MM1.S cells recovered from 3D collagen cultures showed a higher clonogenic potential when compared to 2D cultures.

Conclusion
Here, we established a physiologically relevant model system to co-culture semi-adherent MM cells and MSCs. The collagen-supported 3D co-culture of BM myeloma cells and MSCs might alter their behavior, governing MM cell viability and clonogenicity, in a manner different to conventional 2D systems. Further optimization of the 3D co-culture will provide significant insights in the behavior of MM cells and, ideally, their response to anti-cancer treatments.

keywords: 3D culture, cancer, MSC, co-culture, Multiple Myeloma

62825424005

PRECLINICAL 3D BIOPRINTED MODEL OF OVARIAN CANCER TUMOR MICROENVIRONMENT TO TEST MIRNA-BASED PERSONALIZED THERAPIES

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Epithelial Ovarian cancer (EOC) is the most lethal malignancy in women. Despite de-bulking surgery, chemotherapy and radiotherapy almost 90% of EOC patients will relapse and succumb. One of the main causes of drug resistance and metastasis is the tumor microenvironment (TME) consisting of cancer-associated fibroblasts (CAFs), endothelial cells (ECs), anergic immune cells, adipocyte stem cells (ASC) and other components1,2. Mounting evidence suggest that microRNAs (miRNAs) play critical roles in shaping the TME by inhibiting gene transcription in cancer cells3.
We have previously shown that miR-200c was able to contrast the induction of the immune checkpoint PD-L1, c-myc and β-catenin oncogenes expression by combinatorial therapies in EOC cell lines and biopsies4. Another study showed that miR-200c inhibited epithelial-to-mesenchymal transition (EMT), by targeting ZEB15. Recently, ASCs were discovered as key players in EMT of OC1,6. Since miR-200c targets multiple genes and de-regulates different molecular pathways, more physiologically relevant human preclinical models are required to unravel its role in TME.
In this work, we 3D printed a biomimetic human 3D printed-OCTME model where miR-200c transfected tumor cells interact closely with immune cells and ASCs. The proposed model conveys unprecedent physiological features to better evaluate the effects of miR-200c on i) anti-tumor T-cell response, ii) ASC immunomodulation and iii) EMT in OC cells.
For the 3D TME model, a microfluidic-based extrusion bioprinter was used to deposit two GelMa-based bioinks containing miR-200c-transfected SKOV3 cells and ASCs isolated from healthy donor. The microfluidic spatial control on the material deposition results in the fabrication of cylindrical constructs with radial cell concentration gradient (i.e. tumor cells in the core, ASCs in the shell). After 3 days, PBMCs, isolated from healthy donors, were added on top of the constructs and let for 3 hours to penetrate the gel. The same bioprinting conditions were also used for the parental cell line transfected with the empty vector. Confocal microscopy of cell-labeled constructs at day 5 revealed the formation of a TME exhibiting the physiological architecture found in vivo. ASCs cells surrounded the tumor site enabling the interaction with tumor cells. T-cells traveled across the stromal tissue and reached the tumor site, demonstrating their successful inclusion in the structure. Cells viability and functionality were addressed by live/dead assay and immunostaining of activated T-cells and SKOV3.
The proposed study aims to develop a 3D TME in vitro preclinical model to better understand the interactions between tumor cells, immune cells and ASCs in the presence/absence of miR-200c. Our technology is a proof-of-concept assay toward a precision stratification of EOC patients before undergoing miRNA-based and drug therapies, to test the therapeutic effectiveness and avoid side effects of these treatments.

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keywords: Epithelial Ovarian cancer, microRNA, microfluidic bioprinting, adipose-derived stem cells, engineered tumor microenvironment

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A BIOPRINTED RHABDOMYOSARCOMA MODEL WITH MACROMOLECULAR CROWDING TO STIMULATE EXTRACELLULAR MATRIX PROTEIN DEPOSITION

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Introduction: In recent years tumor microenvironment has been recognized as one of the main actors in cancer relapses and metastasis. Indeed, the crosstalk between malignant cells and the niche, as well as the extracellular matrix (ECM) remodeling, not only favor tumor mass growth, but are also preliminary mechanisms to dissemination. These processes have been mainly investigated in tumors of epithelial origin, for which many 3D models have been produced, while little is still known about sarcoma microenvironment. This is true in particular for rhabdomyosarcoma, the most frequently diagnosed soft tissue sarcoma in pediatric patients. Thus, the development of a suitable 3D model to recapitulate the onset of metastasis, which is mostly driven by an altered ECM deposition, is in need. Macromolecular crowding (MMC) has been shown to enhance the process of ECM deposition. The aim of this work was the application of MMC to rhabdomyosarcoma cell lines. A 2D monolayer culture and a 3D spheroid model were compared with a 3D bioprinted construct.

Methodology: RH30 and RD cell lines were cultured under MMC in 2D in presence of a Ficoll cocktail. MMC was also applied to spheroids produced in ultra-low attachment (ULA) plates. Finally, a 3D bioprinted model was produced using the inkjet printing technology and Matrigel as scaffold, to which also MMC was applied. ECM deposition in these samples was analyzed by immunofluorescence and qRT-PCR, and shape parameters were described for the 3D samples. Results. MMC induced ECM deposition in both cell lines in 2D, which resulted in increased fibronectin signal in immunofluorescence images. MMC-treated spheroids displayed not only augmented ECM protein presence, but also reduced dimensions and higher solidity compared to untreated spheroids. Interestingly, only RH30 spheroids treated with MMC attached to ULA culture surfaces, depositing collagen and fibronectin on the bottom of the culture well. Also, the 3D printed constructs treated with MMC were smaller and more compact than the untreated ones, probably due to the increased amount of ECM components; furthermore, after day 10 of culture small multicellular aggregates detached from the core of these samples, mimicking a dissemination mechanism. This release was less evident in the MMC-treated condition compared to control, but interestingly in both conditions RH30 samples released a higher number of spheroids compared to RD. Of note, CXCR4 levels increased in RH30 samples under MMC stimulation, a gene involved in metastasis promotion.

Conclusions: These observations indicate that MMC stimulate ECM deposition in rhabdomyosarcoma cells and induce a metastatic behavior. As future perspective, the MMC-treated and 3D printed model developed in this work could find applications in drug testing.

keywords: macromolecular crowding, 3D bioprinting, rhabdomyosarcoma

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BIOLOGICAL AND MECHANICAL UNIQUE EXTRACELLULAR MATRIX AMONG DIFFERENT SUBTYPES OF DYSTROPHIC EPIDERMOLYSIS BULLOSA

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Introduction: Dystrophic epidermolysis bullosa (DEB) is a genodermatosis caused by mutations in the COL7A1 gene, in which patients exhibit mechanically fragile skin (1). The underlying mutation determines the clinical phenotype, which can range from a severe condition characterized by widespread blistering associated with the development of chronic wounds-RDEB (recessive subtype) - to a relatively mild disorder associated with localized blistering-DDEB (dominant subtype). To date, the impact of the different COL7A1 mutations on ECM biological and mechanical properties is largely unexplored. Hence, this work aimed to investigate the differences in ECM that occur amongst DEB subtypes and, for the first time, to establish a correlation between these alterations and ECM mechanical properties.

Methodology: Immortalized cells from patients representing three DEB subtypes (different COL7A1 mutations and disease aggressiveness) were obtained from EB House Austria. As a control, healthy primary fibroblasts were used. The cells were cultured for 14 days with 50μg/mL ascorbic acid to promote maximum ECM deposition. Mass spectrometry-based label-free quantification was used to assess changes in the deposited ECM. Western blot and immunocytochemistry were used to further dissect the abnormal ECM features in the different DEB subtypes. Furthermore, the influence of distinct COL7A1 mutations in ECM mechanical properties was addressed at nanoscale by Atomic Force Microscopy (AFM) and at macroscale by uniaxial tensile testing.

Results: Extracellular proteome analysis revealed that fibroblasts from each DEB subtype have their own unique proteomic fingerprint. Independently of the disease subtype - and its associated clinical aggressiveness - down-regulation of structural proteins that impact ECM tensile strength and compression to resistance were identified. Additionally, all DEB subtypes demonstrated a decrease in the dermal-epidermal junctional proteins collagen IV and laminin, as well as an increase in ECM proteins closely associated with wound healing and scarring (tenascin C and vimentin). Furthermore, those differences strongly impact the ECM mechanical properties. At the nanoscale, data indicated a significant reduction in the mechanical stability of the native ECM produced by the different DEB subtypes, which was simultaneously associated with a significant decrease in macroscale stiffness. Interestingly, the more severe subtype (RDEB) results in a significant loss in ECM tensile strength when compared to a healthy control, whereas the milder form (DDEB) shows no difference.

Conclusion: This study demonstrates that different COL7A1 mutations in DEB have a significant impact on the overall dermal ECM features, with structural proteins involved in the mechanical stability being down-expressed and proteins involved in pathological ECM remodeling of wound healing and scarring – hallmarks of DEB - being over-expressed. Furthermore, these changes in the biological features of the ECM have a major effect on the native ECM's mechanical integrity at both the macro and nano scales. Overall, this work contributes to the advancement of DEB knowledge, being the first to correlate alterations in ECM composition with its mechanical properties in disease scenario.

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keywords: Dystrophic epidermolysis bullosa; Extracellular Matrix; COL7A1

S16-1
Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential
Room: S2
(28 Jun 2022, 13:30 - 15:00)

Conveners:
Sylvia Nürnberger; Andrea Barbero

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EXTRACELLULAR MATRIX DERIVED SCAFFOLDS FOR CARTILAGE AND OSTEOCHONDRAL DEFECT REPAIR

Daniel Kelly (Trinity College Dublin, Dublin, Ireland)

Articular cartilage and osteochondral defect repair remain major clinical challenges. Biomaterial scaffolds currently in clinical use in orthopaedic medicine do not accurately mimic native tissues, and therefore do not preferentially promote tissue-specific regeneration when they are colonised by endogenous stem/progenitor cells post implantation. Tissue-specific extracellular matrix (ECM) derived scaffolds have been shown to promote tissue repair by providing both structural and functional cues to cells, suggesting that such natural, biomimetic materials may provide a cell-inductive platform for the regeneration of musculoskeletal tissues. Indeed, decellularized articular cartilage ECM derived scaffolds have been shown to promote the chondrogenic differentiation of mesenchymal stem/stromal cells (MSCs), while bone and growth plate ECM derived scaffolds have been shown to support osteogenesis. However, directing the phenotype of stem/progenitor cells is only the first step in ensuring successful cartilage or osteochondral defect repair following scaffold implantation; such biomaterials are also required to direct the structural organization of the repair tissue to promote functional regeneration. This talk will summarise our efforts towards developing such ECM derived scaffolds for cartilage, bone and osteochondral repair, and will provide preclinical data in relevant in vivo models to support their continued development in the field of orthopaedics.

keywords: Cartilage; Osteochondral; Extracellular Matrix; Scaffold

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CHARACTERIZING IN VIVO DEFORMATION DYNAMICS IN ORGAN SCAFFOLDS USING INTRAVITAL MICROSCOPY

Peter Corridon (Khalifa University, Abu Dhabi, United Arab Emirates), Anousha Khan (Khalifa University, Abu Dhabi, United Arab Emirates)

Introduction: Decellularization creates cell-free collagen-based extracellular matrices from native organs, which can be used as scaffolds for regenerative medicine applications1-8. This technique has gained much attention in recent times. However, there is still a limited understanding of scaffold responses in vivo post-transplantation and ways we can improve scaffold durability to withstand the in vivo environment9. This study uses intravital microscopy (IVM) to gain instant feedback on their structure, function, and deformation dynamics.

Methodology: In vivo assays were developed to evaluate the effectiveness of decellularization and structural and functional integrity of the acellular nephron in the post-transplantation environment. Cohorts of 2-3-month-old male Sprague Dawley rats were used: non-transplanted (n = 4), transplanted day 0 (n = 4), transplanted day 1 (n = 4), transplanted day 2 (n = 4), and transplanted day 7 (n = 4). Qualitative and quantitative assessments of scaffold DNA concentrations, tissue fluorescence signals, structural and functional integrities of various decellularized nephron segments, and velocity within the microcirculation were acquired and compared to the native (non-transplanted) organ.

Results: Large molecular weight dextrans, which lined the vasculature, provided real-time evidence of ischemia onset and microvascular permeability increases. We observed substantial translocation of macromolecules from glomerular/peritubular capillary tracks as early as 12 hours post-transplantation. Blood extravasation continued across a week. During that time, the decellularized microarchitecture was significantly compromised and thrombosed.

Conclusions: Models examining the microvasculature primarily utilize in vitro/in vivo techniques that cannot provide adequate spatial/temporal resolution. These results identifies IVM as a powerful approach for studying scaffold viability and identifying ways to promote scaffold longevity, and angiogenesis in bioartificial organs. We also created the basis to develop a fractal model that can be used to explore ways to improve scaffold integrity to support recellularization and withstand deformation in transplantation environments.

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keywords: intravital microscopy, decellularization, transplantation, organ scaffold

31412705928

DEVELOPMENT AND CHARACTERISATION OF A NOVEL 3D BIOPRINTED BIOMIMETIC COLLAGEN AND HYALURONIC ACID SCAFFOLD FOR THE REPAIR OF CARTILAGE DEFECTS

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Introduction:
Articular cartilage facilitates the frictionless movement of synovial joints, however, due to its avascular and aneural nature, it has limited ability to self-repair. Current treatments for cartilage defects elicit variable results – an issue that the field of tissue engineering has aimed to address; however, the inability to mirror the complexity of native tissue with current biomaterials has hindered progress 1, 2. The advent of 3D-printing has provided a potential solution. 3D-printed (3DP) scaffolds, fabricated using biomaterials native to articular cartilage, can be designed to mimic native articular cartilage. These biomaterial-based printable inks can also be functionalised with cells, bioactive factors and/or gene therapeutics to form biomimetic 'bioinks', capable of repairing cartilage 3.

The aim of this study is to develop a novel 3DP scaffold composed of biomaterials native to human articular cartilage, such as collagen and hyaluronic acid, which can also be incorporated with mesenchymal stem cells (MSCs) and/or therapeutic biomolecules to promote regeneration of the native tissue.

Materials and methods:
To this end, 3.5% neutralised collagen type I was mixed in a 1:1 ratio with methacrylated hyaluronic acid (MeHA) at concentrations of 0.5-3% to formulate four distinct bioinks. The printability of each bioink was first assessed, and three formulations were carried forward to 3D print 10mm x 2mm circular mesh scaffolds. The mechanical and physiochemical properties of the scaffolds were then determined. Two suitable formulations were selected and incorporated with rat MSCs, and the cell viability of the MSCs within 3DP cell-laden scaffolds was determined over 7 days. An optimal bioink formulation was then selected and incorporated with rat MSCs at three respective cell densities. The production of articular cartilage matrix components within these cell-laden 3DP scaffolds was assessed following 21 days culture.

Results and discussion:
Three bioink formulations were found to have desirable 3DP properties and were carried forward for further analysis. Subsequent studies showed no significant difference in the mechanical properties or macro-pore size of scaffolds 3DP with each bioink. However, 3DP scaffolds with a higher concentration of MeHA did have a higher mass swelling ratio. 3DP scaffolds containing the lowest MeHA concentration were excluded from subsequent studies as they could not withstand physical manipulation.

Cell viability studies with the remaining two bioink formulations showed that 3DP scaffolds containing the highest MeHA concentration facilitated greater levels of cell proliferation. Subsequently, this optimal bioink formulation was shown to facilitate deposition of articular cartilage-specific matrix components in 3DP scaffolds in a cell density-dependent manner. Ongoing work includes improving the mechanical properties of the 3DP scaffold by co-printing with a polymer, and incorporation of therapeutic chondrogenic nanoparticles to enhance the

chondrogenic potential of the bioink.

Conclusion:
A biomimetic collagen and hyaluronic acid-based bioink with favourable 3D-printing properties was successfully developed. Scaffolds printed using this bioink facilitated proliferation of MSCs and deposition of new articular cartilage matrix.

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Acknowledgements:
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keywords: Biomaterials, 3D printing, cartilage repair

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DECELLULARISED PLEURAL MEMBRANES IN PULMONARY REGENERATIVE MEDICINE

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Introduction:
Prolonged alveolar air leaks are post-surgical complications to routine lung resections and biopsies that are a significant cause of patient morbidity. Extended duration of chest tube drainage and emergency revision surgeries are the standard approaches for its clinical management. Transplantable decellularised pleural membrane patches as adjuncts to traditional intraoperative closure techniques could reinforce the mechanical barrier, reducing incidence and severity of sustained air leaks. As a treatment modality, it can provide the physiological cues that stimulate endogenous tissue regeneration. We aimed to optimise a decellularisation and characterisation protocol for porcine pleural membranes (PPM), with minimal disruption to the microarchitecture, biochemical composition, and mechanical integrity of the native tissue.

Method:
PPM decellularisation was performed with physical (freeze-thaw cycles) and chemical (0.5% sodium deoxycholate and 1% Triton-X100 in 10mM Tris buffer) treatments. Protocol efficiency was determined via histological analysis (Hematoxylin and Eosin, Alcian blue and Picrosirius red), nuclear membrane integrity (DAPI staining), and quantitative bioassays (Picogreen dsDNA quantification and dimethyl methylene blue (DMMB) glycosaminoglycan assay). Decellularised PPM were assessed for their cytotoxicity (Live-Dead cytotoxicity kit, Invitrogen™, and Trypan blue exclusion assay) and biocompatibility (MeT-5A cell-line seeding and culture). Proteomics was carried out using antibody microarray technology (scioDiscover™, Sciomics GmbH)

Results:
H&E staining of decellularised PPM showed absence of stained nuclei, consistent with significant reduction ($p < 0.0001$) in DAPI stained nuclei counts against native controls. Residual DNA quantification in the decellularized PPM reflected over 90% reduction in native nuclear dsDNA ($p < 0.001$). Staining for sulphated glycosaminoglycans (sGAG) and collagen exhibited minimal disruption to the structural alignment of the native ECM. sGAG content in the decellularised PPM showed a significant reduction in comparison with native controls ($p < 0.01$). Mechanical characterisation studies showed that increased decellularised membrane thickness ($p < 0.05$) did not affect the inherent membrane stiffness, as the estimated Youngs modulus in the decellularised PPM (12782.7 kPa ± 3874) was comparable with the native controls (9259.5 kPa ± 2079). In vitro cytotoxicity and scaffold biocompatibility studies exhibited minimal inhibitory effect on MeT-5A cell line attachment, proliferation, and viability. Proteomics provided molecular readouts of the native and decellularised PPM proteome, reflecting differential protein expressions and enabling decoding of our decellularised PPM matrisome.

Conclusion:
Our pilot study represents a step forward in deriving bioactive ECM scaffolds in the form of decellularised PPM. Studying the recellularisation dynamics of the cell-seeded scaffolds using primary mesothelial cultures will underpin our research towards developing proof of concept for the application of the relatively unexplored decellularised pleural membranes in biological ECM scaffold-based therapeutic approaches.

keywords: Decellularised ECM matrices, Matrisome, Cell-ECM interactions, Tissue engineered therapeutics

62825410577

DESIGNING A PEPTIDE HYDROGEL FOR EARLY DETECTION OF CANCER

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Introduction:
Cancer early detection is pivotal to patient survival. The small non-coding nucleic acid sequences, microRNA (miRNA) are a captivating molecular target for cancer early detection. miRNA are dysregulated during the early stages of cancer1, it is found in stable amounts in blood plasma and serum. Therefore, a minimally invasive liquid biopsy screening device would allow for point of care diagnostics. Current miRNAs detection methods are cumbersome and lack reproducibility along with poor sensitivity and low accuracy. To overcome these challenges we aim to develop a new diagnostic platform using a functional 3D peptide hydrogels for sequence specific2, 3, PCR-free, fluorescent detection of miRNAs in a “one-pot” assay. This work will assess the suitability of the novel hydrogel-based technology for rapid, robust and reliable screening of the unique miRNA fingerprint of difficult to detect cancers.

Experimental methods:
Using a split probe system with a FRET pair conjugated to an anti-parallel β-sheet peptide hydrogel, can be formulated that allow complementary strands of cancer miRNA biomarkers to be identified via fluorescence.
Diffusion characteristics were evaluated via plate reader and Fluoroblok well insert. Allowing to measure the rate at which fluorescently label DNA analogue of miR-21 diffuse into peptide hydrogels.
Cell culture of Panc-1, MIA PaCa-2, LNCaP and PC-3 cells in 2D and 3D (multicellular tumour spheroids) forms was undertaken to validate the sensor. The hydrogels under investigation were also evaluated in their ability to support the four cancer cell lines spheroid structure.

Results & discussion:
Four de novo designed self-assemble peptide hydrogels (SAPH) were tested to understand the diffusion characteristics of miRNA and select system that allow fast trapping and detection of miRNA. MiRNA being negatively charge it was found that positively charted hydrogels promoted miRNA trapping. The mesh size of the hydrogel used (<40nm) also allowed to filter and avoid interference from larger cell debris usually present in biological samples.
The bio-compatibility of the four peptide hydrogels provides a 3D platform for cancer cell culture, and in situ bio-sensing.

Conclusions:
The self-assemble peptide hydrogel is an extremely versatile material. Has the potential to harbour fluorescent properties, to allow for biosensor application in the early detection of cancer.
Future work on 3D culturing of other pancreatic and prostate cancer cells for quantification of the key secreted biomarkers linked to the two cancers that require early detection.

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keywords: Cancer, miRNA, biosensor

62825408605

COLLAGEN/PRISTINE GRAPHENE AS AN ELECTROCONDUCTIVE INTERFACE MATERIAL FOR NEURONAL MEDICAL DEVICE APPLICATIONS

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Introduction
Growing clinical demands for electrical stimulation-based therapies for central nervous system applications requires the development of conductive biomaterials balancing conductivity, biocompatibility, and mechanical performance. Traditional conductive materials often induce scarring, due to their stiffness and poor biocompatibility, hindering their clinical translation and efficacy. To address these issues, we report the development of a pristine graphene-based (pG) composite material consisting of type I collagen and 60 wt% pG, yielding conductivities (~1 S/m) necessary for efficient electrical stimulation, and with versatile processability.

Materials and Methods
Pristine graphene and collagen films (60 wt%, CpG) (CpG60%) were synthesised[2]. Different neurons (SHSY-5Y, NSC-34, iPSC-derived) and glial cells were seeded on the composites, and the metabolic activity, DNA content, cell morphology and release of inflammatory cytokines were assessed. Electrical stimulation was applied to mouse primary cortical neurons to enhance neurite outgrowth and viability. Finally, to demonstrate the versatility of CpG composites for a number of applications, the CpG was fabricated into porous 3D scaffolds, microneedle arrays, and bioelectronics circuits, using freeze drying, dry casting, and 3D printing approaches respectively.

Results
Of all composites tested (N=4), CpG60% exhibited physiologically relevant conductivities (~1 S/m), and robust mechanical properties (~17.8 MPa). Four neuronal and glial cell types exhibited robust growth when grown on composite films with no change in inflammatory markers IL-6, IL-10, or IL-1β, and good biocompatibility. Induced pluripotent stem cell-derived neurons exhibited typical cellular morphology after 15 days growth on the films. The achieved conductivity enabled the efficient delivery of electrical stimulation to mouse primary cortical neurons on the composite (200mV/mm, 12Hz, 4h/day, 5 days), and enhanced neurite outgrowth, cellular viability and morphology compared to collagen controls. Finally, the diverse potential applications of the composite were demonstrated using a range of neural-interfacing structures, including porous scaffolds with aligned pores visible under SEM, microneedle arrays, and 3D-printed working LED circuits for bioelectronics.

Discussion
These results show that (CpG60%) composites form a versatile neurotrophic platform that balances the requirements for physiologically relevant conductivity, robust mechanical properties, and excellent biocompatibility. The mechanical properties of the composite give it an advantage over stiffer traditional electrode materials, which can cause scarring due to extreme mechanical mismatch. The CpG60% composite supported robust neuronal and glial cell growth, with an absence of neuro-inflammatory responses. In addition, CpG60% efficiently delivered electrical stimulation to neurons, which when coupled with these conductive materials enhanced

neurite outgrowth, viability, and cellular morphology. Finally, the versatile processing capabilities of the CpG composites using various fabrication techniques demonstrate its potential as platform for fabrication of next-generation neuronal medical devices.

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keywords: tissue engineering, nanomaterials, nerve repair, graphene, electroconductive

83767225884

NOVEL HYPOXIA MIMICKING PEG-BASED NANO-BIOINK FOR CARTILAGE REGENERATION APPLICATION

Subhashini Ravi (Indian Institute of Technology Hyderabad, Hyderabad, India)

By its avascular nature and limited healing potential, articular cartilage (AC) defects are still challenging to cure, resulting in degenerative diseases such as osteoarthritis. Several clinical techniques aim to repair the AC; however, load-bearing and fully functional tissue recapitulation remain a significant hurdle. In the last few decades, tissue engineering has given hope for resolving the issues associated with the existing therapy methods. Essential innovations in 3D bioprinting technology have led to a greater focus on successfully implementing engineered tissue constructs. For cartilage regeneration, Mesenchymal stem cells (MSCs) are a potential cell source in a unique milieu known as the stem-cell niche, characterized by low oxygen levels. Cobalt is well known for its hypoxic effects in vitro by stabilizing hypoxia-inducible factor (HIF-1α), a central regulator of stem cell fate. The main aim of this study was to evaluate the impact of Cobalt nanowires (Co NWS) on the chondrogenic potential of human umbilical cord MSCs (UMSCs) encapsulated in the PEGDA/Alginate hydrogel. In the current study, cell proliferation, mechanical properties, and the expression of chondrogenic markers were analyzed. Co NWS supplementation into the PEGDA/Alginate hydrogel enhanced the cell proliferation and mechanical properties and showed the upregulation of chondrogenic markers such as SOX 9, COL2A1, and ACAN through the HIF-1α pathway. Together these findings are taken into consideration the potential of hypoxia mimicking hydrogels in the treatment of osteoarthritis.

keywords: Cobalt nanowire, Hypoxia, HIF 1-α, Cartilage tissue engineering

41883642808

A SCAFFOLD-FREE GRAFT FOR LARGE CRITICAL SIZE BONE DEFECT: PRECLINICAL EVIDENCE TO CLINICAL PROOF OF CONCEPT

Nicolas Theys (Novadip Biosciences, Mont-Saint-Guibert, Belgium), Pierre-louis Docquier (Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium), Denis Dufrane (Novadip Biosciences, MONT-SAINT-GUIBERT, Belgium)

Introduction. Large critical size bone defect is one of the most challenging pathologies in orthopaedic surgery. NVD-003 is an autologous scaffold-free cell-based osteogenic implant intended to improve bone healing in severe pathophysiological conditions. This study aims to investigate the therapeutic potential of NVD-003, an osteogenic graft derived from human adipose stem cells.

Methods. NVD-003 consists of autologous osteogenic cells cultured from adipose tissue derived stem cells embedded in their extracellular matrix with hydroxyapatite/beta-tricalcium phosphate (HA/TCP) particles. The bioactivity of NVD-003 was studied in nude rat models: (i) to compare the impact of fresh or decellularized grafts in term of angiogenesis (up to 1 month) in a fibrotic tissue (in a cauterized muscular pocket); (ii) to assess its in vivo osteogenicity (in comparison to HA/TCP particles alone), at 1/2/3 months post-implantation, in an irreversible femoral critical size bone defect. The angiogenesis was quantified by histomorphometry while the osteogenesis was studied by micro-CTscan, histomorphometry and Q-RT-PCR on graft explants. Four paediatric patients (5 to 15 years old) suffering from a congenital pseudarthrosis of the tibia were treated in a compassionate use program. Three months after the adipose tissue procurement, the 3D-grafts were placed into the defect and followed clinically and radiologically.

Results. (i)- Preclinical: After 1-mo of intra-muscular implantation, cellular survival of human cells and the promotion of angiogenesis were observed. The number of blood vessels number per tissue area at 12 weeks post-implantation and the blood vessels area per tissue area at 8 weeks post-implantation were noticeably increased in NVD-003 implants as compared to HA/TCP particles. Quantitative analysis of µCT images at successive imaging time points (4, 8 and 12 weeks) showed that a similar level of mineralization was observed at each time point, indicating the absence of resorption of the test item up to 3 months post-implantation. A complete integration and bone fusion were found for the 3D graft in comparison to HA/TCP alone which revealed a lack of tissue remodelling and osteogenesis. Specific genes of the skeletal development were overexpressed in the bone defect treated with the NVD-003 (at 4/8 weeks post-implantation) while no osteoinduction was found for the HA/TCP particles alone. Changes in bone formation were assessed using histomorphometry measurements of the entire implant site. Bone area showed a trend towards an increase with NVD-003 already at 8 weeks post-implantation, while a noticeable increase in bone area was observed with NVD-003 as compared to HA/βTCP samples at 12 weeks post-implantation.
(ii) Clinical: A large volume (>15cm3) of the autologous 3D graft was manufactured in aseptic areas under GMP requirements for each patient and then implanted without any modification of the surgical procedure. The graft was easily handled, shaped, and implanted. NVD-003 implant demonstrated a continuous remodelling (with bone formation) up to 14 months post-implantation to obtain a sufficient bone fusion (allowing walk without pain) and no recurrence of the disease.

Conclusion. The NVD-003 graft plays a major role to induce angio- and osteogenesis in a

complex environment and to recover a bone fusion in a critical-sized bone defect.

keywords: stem cells, bone, congenital pseudoarthrosis, tissue engineering

S16-2
Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential
Room: S2
(28 Jun 2022, 15:30 - 17:00)

Conveners:
Andrea Barbero; Sylvia Nürnberger

41883636486

WHEY PROTEIN ISOLATE: A MULTIFUNCTIONAL DAIRY-DERIVED BIOMATERIAL

Timothy Douglas (Lancaster University, Lancaster, United Kingdom)

Whey protein isolate (WPI) is an inexpensive byproduct from the dairy industry which can be processed into autoclavable hydrogels with high compressive strength which support the adhesion, growth and differentiation of cells which can be applied in bone regeneration. Furthermore, WPI hydrogels can solubulized hydrophobic molecules and hence serve as a delivery vehicule for hydrophobic molecules with biological activity (e.g. antibacterial agents.) WPI can also be processed into flibrillar coatings for biomaterials, which also support the adhesion, growth and differentiation of cells. This presentation will review the aforementioned applications of WPI.

keywords: Hydrogel, coating, bone, carrier, drug delivery

41883601764

HYDROLYTIC DEGRADATION CHARACTERIZATION OF 3D PRINTED POLYESTER SCAFFOLDS UNDER STATIC CONDITIONS AND FLOW PERFUSION

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INTRODUCTION
Creating biofunctional scaffolds could potentially meet the demand for patients suffering from bone defects without having to rely on donors or autologous transplantation. 3D printing has emerged as a promising tool to fabricate scaffolds with high precision and accuracy by computer design using patient-specific anatomical data1. Among other relevant key points for 3D-printed bone scaffold selection, to achieve controlled degradation profiles is an essential feature to consider. Thus, the importance of a deep characterization of the biomaterial degradation under physiological conditions is needed2.

METHODOLOGY
50:50 blend made of PCL-PLGA was created to fabricate cylindrical scaffolds by 3D printing. The blend was fabricated by dissolving PCL pellets and PLGA powders in dichloromethane, by casting and evaporating the solvent. PCL-PLGA filaments were extruded with a mechanical extruder. Cylindrical scaffolds were finally printed with a 7mm diameter, 2mm height, 400µm pore sizes. Their hydrolytic degradation under different conditions was quantified.
Static buffer medium and flow perfusion were applied to the samples inside an in-house fabricated bioreactor, which contains four main individual chambers. Perfusion tests were done inside the bioreactor thanks to a roller pump which imposed a PBS flow rate of 4 mL/min. Samples were incubated in normoxia (21% O_2 and 5% CO_2) for two and four weeks. Degradation under static conditions was also conducted inside the bioreactor with no flow. During both conditions, PBS in the wells was exchanged every two days and the pH was measured periodically.
Several techniques were used to characterize the degradation of the polymers by the end of the incubation period including chemical changes on the surface by x-ray photoelectron spectroscopy, polydispersity index by gel permeation chromatography, surface inspection by scanning electron microscopy, mechanical properties decrease weight loss and medium acidification over time.

RESULTS AND CONCLUSIONS
In this work, we have thoroughly characterized the hydrolytic degradation of the final samples at different incubation periods, achieving different outcomes in agreement with our initial hypotheses. Results confirm a faster degradation of PCL-PLGA scaffolds when flow is forced through the samples. Besides, it was also confirmed the quicker degradation of PLGA in the blend. In addition, time is also a key factor and we obtained significant differences for both incubation times: 2 and 4 weeks.

ACKNOWLEDGEMENTS
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keywords: Bone tissue engineering, polymeric scaffold, hydrolysis, polyester, 3D printing

20941818306

FIBRIN-BASED HYDROGELS WITH TUNEABLE MECHANICAL PROPERTIES

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Introduction
In present times, implant development focusses on optimizing biocompatibility, mechanical strength, and reproducibility. Although cardiovascular implants currently available on the market are medically established and therefore widely used, they have a limited lifetime which also impacts the mechanical properties and functionality. Additional issues are the formation of blood clots in mechanical implants (heart valves), the formation of bacterial biofilms (dental implants), and the degradation of bioprotheses. Also, implant rejection by the patient's body is still a challenge. A solution for these issues can be the use of biohybrid hydrogels. Especially fibrin-based hydrogels are very promising.

Methodology
The aim of this research is the synthesis and characterization of fibrin-based hydrogel-matrices for Tissue Engineering applications with the research focus on cardiovascular implants, to be precise on heart valve replacements. We have reinforced fibrin by the addition of linear functional copolymers, specific fibrin-binding peptides, and functional microgels. Our goal is to establish an innovative functional tool-box for fibrin-based biohybrid hydrogels that allow also for patient-specific individualisation of implants. The effect of incorporated functional additives in the hydrogel-matrices is analyzed in regard to their mechanical properties, fiber-network morphology and biocompatibility.

Results
In terms of our research, we could already demonstrate that the addition of linear poly(N-vinylpyrrolidone)-copolymers with functional epoxide groups can enhance the mechanical behavior of the fibrin-based hydrogels due to covalent crosslinking, resulting in higher storage moduli, thicker fibers, and a decreased degradation rate compared to pure fibrin-hydrogels. The obtained hydrogels additionally possess a high biocompatibility as proven by cell viability experiments.

In addition, specific fibrin-binding peptides were applied, which exhibit supramolecular interactions within the fibrin-matrix. A combination of supramolecular and covalent interactions by mixing linear polymers and fibrin-binding peptides in various ratios can enhance the strain-stiffening behavior of the hydrogel matrix. Also, the fiber thickness could be increased.

As a third modification of fibrin-based hydrogels, functional thermoresponsive microgels were used instead of linear copolymers and specific peptides. Just like the linear copolymers, the N-vinylcaprolactam-based microgels include glycidyl methacrylate as a functional comonomer to enable covalent attachment to the fibrin by epoxide groups. We can demonstrate that the use of microgels as colloidal crosslinkers resultes in hydrogels providing a temperature-dependent increase in storage modulus, which is not present in pure fibrin-gels. As microgels are widely studied for their possible application in drug delivery, owing to their ability to encapsulate active substances, their use is of high interest in Tissue Engineering applications.

Conclusion
We could develop a functional tool-box for the reinforcement of fibrin-based hydrogels. The mechanical and morphological properties can be tailor-made by selecting the respective type of additive. Regarding the Tissue Engineering of materials mimicking native heart valves, compartments with different mechanical properties are needed, which is exactly what our tool-box allows us to create.

keywords: Hydrogels, Fibrin, Microgels, Tissue Engineering

83767224905

IMPROVED CELLULAR INFILTRATION BY GLYCOSAMINOGLYCANS REMOVAL AND ALTERED STIFFNESS - A STUDY ON AURICULAR CARTILAGE SCAFFOLDS.

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Introduction
Cell infiltration is essential for the repopulation of dense materials in tissue engineering. During that process, several factors, such as scaffold topography, mechanical properties or porosity play a key role. These acquire special importance when designing the materials to substitute and regenerate articular cartilage. Because of their similar structure and composition, decellularized cartilage scaffolds represent an ideal candidate. Our group previously developed a biomaterial from auricular cartilage, which allowed us to study cell migration [1]. Removing the elastic fibres and depleting the glycosaminoglycans (GAGs) leaves a network of open channels. This process reduces the matrix density, altering their mechanical properties and enhances the elastic fibre removal process. In this study, we aimed to investigate the influence of GAG removal on the mechanical properties and cellular ingrowth on decellularized auricular cartilage scaffolds.

Methodology
Scaffolds were harvested from bovine ears by punching 8mm diameter discs as described previously [1]. Briefly, scaffolds were cut to a standard thickness and subjected to several freezing-thawing cycles. Afterwards, an enzymatic treatment comprised of pepsin and elastase was used. Because pepsin is the enzyme that removes the GAGs, a concentration series from 0 to 1 mg/mL was used. Elastase, the enzyme that depletes the elastic fibres was maintained constant at a concentration of 0.03 U/mL. First, GAG content was quantified by histology (Alcian blue staining) and blyscan assay. Second, fluorescent-labelled adipose-derived stromal cells (ASCs) and human articular chondrocytes were seeded on the scaffolds and cultured under dynamic conditions for one week. Cell infiltration was monitored by confocal microscopy and immunohistochemistry. Furthermore, the mechanical properties were measured by a stepwise compressive test using a Zwick uniaxial testing machine. The viscosity and elasticity of the samples were further computed using a mathematical model. For comparison of two groups unpaired t-test or Mann–Whitney test was chosen according to distribution.

Results
A concentration series of pepsin was used to modify the GAG content, which was inversely correlated. Residual GAG concentration after high and low pepsin concentrations differered

significantly (1 mg/mL pepsin: 0.058 0.014 mg/µg vs. 0.2 mg/mL pepsin: 0.15 0.042 mg/µg; p = 0.0022). Cellular infiltration, however, followed the inverse trend. Higher concentrations of pepsin increased cellular infiltration. Nevertheless, even the lowest tested concentration (0.2 mg/mL) maintained adequate levels of cell migration into the open channels. Interestingly, cells were unable to penetrate the scaffolds treated without pepsin, forming a monolayer on the surface. In the slower infiltrating chondrocytes, the effect of matrix treatment was even more visible than for ASCs. Additionally, the mechanical properties followed a similar pattern. Native scaffolds and those treated with 0.2 mg/mL pepsin showed a viscoelastic behaviour and higher stiffness. On the contrary, concentrations above 0.4 mg/mL led to a more viscoplastic and fluid-like behaviour.

Conclusion
Auricular cartilage scaffolds are a suitable tool to study cell infiltration. Pepsin reduces considerably the GAG content, leading to reduced stiffness. Cell migration into the scaffolds is highly dependent on both parameters, which are crucial for scaffold development.

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keywords: glycosaminoglycans, stiffness, cell infiltration

73296328205

THE PREPARATION AND CHARACTERISATION OF POLY(3-HYDROXYBUTYRATE-CO-4-HYDROXYBUTYRATE) [P(3HB-CO-4HB)] BASED BIOCOMPOSITE FOR TRANSLATIONAL BIOMEDICAL APPLICATIONS

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Introduction:
The development of novel biocomposite formulations has significantly contributed to the biomedical field- specifically within translational and clinical applications. This study centres on the polyhydroxyalkanoates-based copolyester superfamily of materials, with a particular emphasis on the poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] copolymer as a novel biomimetic substrate. Both 3HB and 4HB monomers have been recognised as natural metabolites in mammalian systems and this copolymer can be synthesised using a microbial-based production system. Crucially, it has been accepted that the 4HB monomer molar fraction determines the physical characteristics of the final copolymer which, ultimately, defines its endpoint application. This project compares two different fermentation techniques used for the synthesis of P(3HB-co-4HB) in terms of yield, purity and composition. Importantly, the synthesised material is characterised in terms of its physical, mechanical and biological profiles before being further enhanced/functionalised with 45S5 bioactive glass and graphene.

Methodology:
P(3HB-co-4HB) copolymer, from Cupriavidus sp. USM1020, was extracted from a shake flask cultivation or bioreactor inoculation system before being purified to obtain crude copolymer. 45S5 bioactive glass was prepared using a sol-gel technique [ratio of 45SiO2-24.5CaO-24.5NaO-6P2O5 (wt%) precursors]. Graphene monolayers were fabricated using liquid exfoliation of graphite. The biocomposite mixture was prepared via facile blending by dissolving P(3HB-co-4HB) crude copolymer in chloroform followed by ultrasonication with the bioactive glass and graphene. Casting was achieved by either solvent or emulsion freeze drying techniques to assess the differences in their porosity. Characterisation was performed using SEM, FT-IR and DSC. Disc diffusion antibacterial assay was employed with Escherichia coli and Staphylococcus aureus. Biocompatibility was assessed using human dermal fibroblasts (HDF) and murine osteoblastic (MC3T3-E1) cells, and standard cell culture assays: cell attachment (SEM imaging, live/dead), proliferation and viability (metabolic activity/MTS, LDH-release), and differentiation activities (cytokine expression/western blotting). Preliminary in vivo studies were performed using male Sprague-Dawley rats.

Results:
The shake flask cultivation system generated a higher percentage (~69%) of 4HB compared to the bioreactor system. Miscibility of the biocomposite was further improved via facile blending. Morphological analyses showed that the emulsion freeze drying technique resulted in a more porous structure compared to solvent casting and the associated change in wettability confirmed with water contact angle measurements. The mechanical profiles of the biocomposite and antibacterial activities were enhanced following incorporation of graphene. Optimised composition of both bioactive glass and graphene within the biocomposite is vital in ensuring optimal level of cells adhesion, which resulted from the observed attachment and proliferation of both cell lines. The animal studies (i.e. skin flap and bone defect) demonstrated good biocompatibility and favour interaction between P(3HB-co-4HB)-bioactive glass-graphene biocomposite and native tissues with enhanced presence of nuclei and neo-vascularisation, and minimal immune response.

Conclusions:
Taken together, this study illustrates the crucial optimisation parameters of the novel formulation P(3HB-co-4HB)-bioactive glass-graphene, which includes processing techniques that affects the final morphology and behaviour of the biocomposite. Owing to the individual benefits of each prominent material utilised in this study, increased potential in translational biomedical applications especially as therapeutic dressings and non-load bearing scaffold for bone regeneration can be considered.

keywords: Biocomposite, Skin, Bone, In vitro

20941865528

HUMAN EPIDERMAL SKIN EQUIVALENTS

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INTRODUCTION:
Extracellular matrix (ECM) protein is often used in cell culture to provide developmental cues for cells such as skin cells. This is especially important for the generation of epidermal models as without a dermal compartment the keratinocytes are reliant on the ECM coating to provide them with a foundation to adhere to and for pro-developmental cues. A major limitation of most commercially available ECM is its composition, as the majority of commercial/scientific suppliers only provide a single ECM component (such as Collagen I). This is a major limitation as this confers reduced physiological relevance in comparison to native human ECM which contains a plethora of ECM components. In addition, commercially available products are often not of human origin which severely limits their physiological relevance and therefore their overall effectiveness in cell culture. As a result, we have developed a methodology of obtaining and purifying a broad-spectrum ECM derived from primary human fibroblasts, which will confer numerous benefits not only to cell culture, but also for topical cosmetic applications and wound healing.

METHODOLOGY:
Primary human fibroblasts were expanded and seeded into a microporous Alvetex® scaffold, which provides a 3D microenvironment to allow for replication of the native conditions found in skin that serves to promote increased cells attachment and ECM deposition. In order to stimulate maximum ECM production, the skin models were cultured in a 54mm diameter Alvetex® scaffold with Dulbecco's modified eagle medium (DMEM) with 10% non-heat treated foetal bovine serum (NHT-FBS). Once the dermal model had reached maturity after 4-weeks of culture time, the ECM was extracted from our in-house generated dermal models by a process of mechanical homogenisation of the dermis followed by re-solubilisation of the extracted ECM components using an extraction solution. This was followed by centrifugation to remove any pieces of the supporting scaffold and cellular debris/insoluble components, leaving the ECM suspended in the supernatant, which was then collected and lyophilized to produce the final purified ECM product.

RESULTS:
Analysis of extracted ECM by Western blotting and total collagen assay showed that the composition of extracted ECM contained predominantly Collagen type I as expected from native human skin. The purified ECM was then used as a coating solution for the generation of epidermal models using primary human keratinocytes in comparison to a range of currently commercially available coating matrixes It was found that this solution was capable of supporting the growth and development of a complex and stratified epidermis.

CONCLUSIONS:
Extraction of ECM from our in-house generated dermal models has been shown to be a viable method for the production of physiologically relevant human derived ECM that is capable of

supporting the growth and development of a multi-layered and stratified epidermis. Overall, this has implications in cell culture model generation to provide more physiologically relevant cell culture conditions and developmental cues. Including further uses in downstream applications in the cosmetic industry whereby human collagen can be incorporated into topical cosmetics to help improve the appearance of skin aging.

keywords: Extracellular matrix, Epidermal model, Bioengineering

62825420164

PRODUCTION OF HIGHLY ANGIOGENIC HYDROGELS FROM THE EXTRACELLULAR MATRIX OF CULTURED STROMAL VASCULAR FRACTION OF ADIPOSE TISSUE

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Introduction Tissue engineering (TE) is an interdisciplinary field that creates functional biologic substitutes for the repair of damaged tissues or organs. One major challenge when generating a functional TE model is its vascularization. Indeed, nutrient and oxygen supply, as well as metabolic waste products collection, are essential for the survival of the engineered tissue after transplantation. Hence, the development of a stable and functional vascular network within the constructs is essential. Using materials that include angiogenic cues as scaffolds for TE constructs may be a potential solution. Understanding this, the stromal vascular fraction (SVF) of adipose tissue has been proposed as a tool for in vitro pre-vascularization1. SVF is a heterogeneous cell population that has shown spontaneous vasculogenesis when cultured in vitro, in the absence of added growth factors. The extracellular matrix (ECM) produced by SVF cells is a key component to this capability. Many recent reports detail the use of ECM-derived hydrogels as TE scaffolds able to support cellular activities due to their similarity to native tissue's ECM. We herein report the development of an angiogenic hydrogel derived from the ECM of SVF cell sheets.
Methodology SVF cell sheets were subjected to a decellularization protocol by a combination of freeze-thaw cycles and a nuclease treatment. Then, the samples were freeze-dried and digested with an acidic pepsin solution, and hydrogel polymerization occurred after pH neutralization with 0.5 M NaOH and temperature increase up to 37ºC for 1h. DNA quantification allowed to assess decellularization efficiency, while circular dichroism (CD) allowed to verify protein secondary structure maintenance. Regarding the ECM-related protein content, SDS-PAGE and Western blot were used. ECM-derived hydrogels were also stained for the presence of nuclei and collagen by using, respectively, Hematoxylin and Eosin (H&E) and Sirius Red/Fast Green staining.
Results DNA quantification and H&E staining confirmed decellularization efficiency. Through CD technique, it was possible to detect the triple helix conformational structure typical of collagen, confirming conservation of protein structure after the extraction protocol. Protein analysis with SDS-PAGE and Western blot revealed high protein variety within the ECM extract, with type I collagen being the predominant one. This was also verified by Sirius Red/Fast Green staining.
Conclusions Overall, these results show that we were able to isolate ECM proteins from SVF cell sheets and successfully create an ECM-like hydrogel with a Freytes solubilization protocol. Ongoing studies are focused on the proteomic characterization of the hydrogel as well as on in

vitro cell culture studies to confirm the angiogenic potential compared with commercial collagen hydrogels. If effective, the use and development of regenerative strategies based on angiogenic ECM-like hydrogels can lead to promising advances in the TE and regenerative medicine fields.

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1. Costa, M. et al., Acta Biomater. 55, 131–143 (2017)

keywords: Vascularization, Tissue Engineering, Extracellular Matrix, Hydrogels

94238130728

HOW NATURAL BIOMATERIAL CONSISTENCY LEADS TO PREDICTABILITY AND TUNABILITY

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Introduction: Due to their biocompatibility, biodegradability and cell-interactivity, gelatins are widely used in the biomedical and tissue engineering fields. Gelatin can be chemically crosslinked to originate constructs that are stable at body temperature. However, if modified gelatins cannot be produced with consistent molar mass and degree of modification (DoM), these advantageous biomaterials cannot be used in a reliable manner. To achieve predictable hydrogel strengths, consistent GelMA molar mass, degree of modification and the associated photo-kinetics need and can be tightly controlled.

Methodology: Six GelMA types (90 kDa and 160 kDa at DoM of 40, 60 and 80 %), were dissolved at concentrations of 5, 10 and 15 w/v% in PBS. The photo-initiator LAP was added to the GelMA resins. The photo-crosslinking kinetics of the GelMA resins were studied at 20 °C using a TA Discovery Hybrid Rheometer HR-2 (TA Instruments, the Netherlands) that was equipped with an Hönle - Bluepoint 4 - UV-A light (7.4 mW cm-2). The samples were in situ irradiated from the bottom through the quartz plate using a parallel plate setup (Ø 20 mm, gap 0.300 mm). A shear frequency of 1 Hz and an amplitude (strain) of 1 % were selected as they were within the linear visco-elastic range of the GelMA materials. The storage moduli (G’) were monitored over time.

Results: The herein presented data confirm that with increasing degree of modification (DoM) the storage modulus of the GelMA hydrogels will increase too. The increase in DoM can be correlated to an increase in storage modulus with an exponential component of e~0.6x for the 160 kDa GelMAs and an exponential component of e~0.54x for the 90 kDa GelMAs. With increasing GelMA concentrations (w/v%) the storage modulus of the hydrogel will increase. This statement was confirmed for the 90p GelMAs with a near perfect power-correlation (x~3.3). Interestingly, the 160 kDa GelMAs do not show a power correlation, but a linear one, between hydrogel strength and GelMA concentration. As a result of these different correlations, a convergence is seen between molar mass affected storage modulus, and concentration (w/v%) affected storage modulus. At low GelMA concentrations a clear difference is seen between molar mass and storage modulus, but as concentration increase this difference in storage moduli becomes smaller and smaller.

Conclusion: With consistent GelMA molar mass and degree of modification, hydrogel strength becomes predictable, as evidenced by the exponential-correlations found for the 160 kDa GelMA (e~0.6x) and 90 kDa GelMA (e~0.54x). Regarding hydrogel strength in function of GelMA concentration, a power-correlation was evinced (x~3.3) for 90 kDa GelMAs and a linear correlation for 160 kDa GelMAs, showing a convergence in hydrogel strength across GelMA molar mass and GelMA concentration.

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keywords: GelMA, hydrogels, predictability, tuneability, biofabrication

S17
Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility
Room: S3 B
(28 Jun 2022, 13:30 - 15:00)

Conveners:
Aleksandra Klimczak

FROM GEOMETRICAL PATTERNS TO BIOINSPIRED TOPOGRAPHIES: NANOFIBRILLAR MICROBUNDLES INDUCE STRONG TOPOLOGICAL MODULATION OF PRIMARY HUMAN IMMUNE CELLS

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Supplement-free induction of macrophage polarization solely through the topography of materials is an auspicious strategy but has so far significantly lacked behind the efficiency and intensity of media-supplementation based proto-cols. We investigated Melt-Electrowriting (MEW) for the fabrication of fibrous 3D scaffolds made from poly(ε-caprolactone) (PCL) and advanced the precisely defined inter-fiber spacing from 100 µm down to 40 µm for a variety of pore geometries (rectangular, triangular and round) with the aim to identify structural design criteria for the fabrication of scaffolds with strong topographic immunomodulation for human monocyte-derived macrophages. These scaffolds did facilitate primary human macrophage differentiation towards the M2 type, which was most pronounced for box-shaped pores with 40 µm inter-fiber spacing, but not with the desired efficiency [1].
We then found that human monocyte-derived macrophages show a strong M2a like pro-healing polarization when cultured on type I rat-tail collagen fibers but not on collagen I films. Therefore, we hypothesized that highly aligned nanofibrils also of synthetic polymers, if packed into larger bundles in 3D topographical biomimetic similarity to native collagen I fibers, would induce a localized macrophage polarization. Through integration of flow directed polymer phase separation into MEW we developed Melt-Electrofibrillation, a process that yields nano-fiber bundles with a remarkable structural similarity to native collagen I fibers, particularly for medical grade PCL. These biomimetic fibrillar structures indeed induce a pronounced elongation of human monocyte-derived macrophages and unprecedentedly triggered their M2-like polarization similar in efficacy as IL-4 cytokine treatment [2].

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94238155587

CELL MEMBRANE CAMOUFLAGE MESOPOROUS BIOACTIVE GLASS NANOPARTICLES EMBEDDING GLUCOSE OXIDASE FOR TARGETED ENHANCED TUMOR THERAPY

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INTRODUCTION: Mesoporous bioactive glass nanoparticles (MBG NPs) have received a significant amount of interest for their potential to treat cancer cells with high drug loading capacity and excellent stability, providing a controlled drug release platform into the malignant tumor [1]. Although enhanced permeability and retention (EPR) effects allow MBG NPs to accumulate at tumor sites, the inferior tumor targeting and ineffective immune escape performance of MBG NPs remain significant challenges. Herein, the tumor targeting and immune escape properties of MBG NPs that were camouflaged with tumor-derived cell membrane and macrophage-derived cell membrane were initially evaluated in vitro and in vivo. And the macrophage cell membrane bionic modified MBG NPs embedding glucose oxidase (QO) drug delivery system (RAW@MBG@QO DDS) was constructed based on its superior performance in immune escape. Finally, we systematically investigated the tumor treatment effect and biosafety of RAW@MBG@QO DDS in order to expand the clinical application of MBG NPs as a drug delivery.

METHODS: MBG NPs were synthesized by a sol-gel method combined with a hydrothermal reaction. For cell membrane camouflage, 4T1 cells and RAW 264.7 cells were separately suspended in a hypotonic lysing buffer and then disrupted using a Dounce homogenizer. The homogenized solution was secondary centrifuged. The two kinds of membranes were collected and mixed with MBG NPs under the sonication induced assembly, respectively. The tumor target and immune escape performance of both cytomembrane coated MBG NPs were analyzed using the in-vitro cell model and the in-situ tumor model, and the RAW 264.7 cells were filtered for developing RAW@MBG@QO DDS. The cytotoxicity, oxidative stress, and tumor suppressive effects mediated by RAW@MBG@QO DDS were further investigated.

RESULTS & DISCUSSION: In the present study, we have successfully constructed two kinds of cytomembrane coated MBG NPs, both of which showed significantly higher tumor targeting compared to MBG. MBG NPs coated with macrophage membrane were more effective at avoiding being phagocytosed and eliminated by the immune cells. Following that, our results indicated that RAW@MBG@QO DDS could release QOs in a controlled fashion to induce the oxidative stress and apoptosis on tumor cells using the catalytic reaction of glucose oxidase, and showed excellent tumor suppressive effect in vivo. Meanwhile, RAW@MBG@QO DDS had the advantage of QO-controlled release, which effectively mitigated blood homeostasis interference and avoided potential toxicological risks.

CONCLUSIONS: Our findings suggest that the cell membrane camouflage MBG NPs embedding

glucose oxidase drug delivery system exhibits high efficiency for tumor suppression, which can promote the development of MBG NPs serving as as excellent drug delivery vehicles for cancer therapy.

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keywords: Mesoporous Bioactive Glass, Cell membrane camouflage, Tumor suppression

83767238355

COMBINING BIOPRINTING AND MELT-ELECTROWRITING TECHNIQUES IN A MULTI-MATERIAL APPROACH FOR THE REPLACEMENT OF THE TEMPOROMANDIBULAR JOINT

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The reconstruction and replacement of musculoskeletal tissues have been extensively investigated in the last decades. Trauma injuries and degenerative diseases are the most common causes reported worldwide. Stem cells play an important role in tissue regeneration and have been successfully applied in musculoskeletal research, especially due to the low self-repair capability of tendons, ligaments, and joints. Due to the specialized architecture of native musculoskeletal components, mostly related to the complex interplay between multiple tissues, interface regions between hard (bone) and connective tissues (cartilage) are challenging to be engineered. When it occurs, they often fail during implantation due to the lack of appropriate mechanical properties. This challenge is even more exacerbated in the temporomandibular joint (TMJ), which is composed of several anatomical structures such as the articular disc, jaw, mandible, muscles, and tendons that connect the scapula, sternum, and neck. The TMJ performs complex movements under compression and tension during common activities such as talking, chewing, and biting. In this context, multi-material approaches that combine different manufacturing techniques can be very promising for interfacial tissue engineering of the TMJ. Hence, the objective of this work was to evaluate the integration of polycaprolactone-polylactic acid (PCL-LA copolymer) fibrous scaffolds produced by melt-electrowriting (MEW) with bioprinted constructs made of xanthan gum (XG) hydrogel and mesenchymal stem cells. MEW meshes were manufactured at 10 mm/s, 170 °C, 0.8 bar of pressure, 6 kV and 4 mm of height. Four-layered constructs were bioprinted varying speed from 40-60 mm/s and pressure from 50-70 KPa using smooth flow tapered tip. Morphological aspects regarding filaments size and porosity of both manufacture techniques were quantified through optical and scanning electron microscopes. Stability in culture media for 28 days was also analyzed. Regular and well-defined PCL-LA meshes were obtained using MEW. Constructs with satisfactory shape fidelity were also obtained through bioprinting. To analyze the most appropriate strategy to improve integration, stability, and mechanical properties between PCL-LA meshes and XG bioprinted constructs, a double crosslinking network has been investigated. First, ionic crosslinking of XG using trivalent iron ions, followed by a photocrosslinking step using acrylate groups in MEW meshes. Overall, based on the hybridization between both processing techniques, employing a multi-material approach, as well as including a double crosslinking strategy we hypothesize that promising interfacial tissues with improved mechanical properties can be obtained. The potential application of the multi-material herein explored are analyzed as a replacement for the multi-tissue temporomandibular joint.

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keywords: bioprinting; melt-electrowriting; stem cells; temporomandibular joint; tissue engineering

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PREDICTION OF IN VITRO SCAFFOLD LIFETIME THROUGH THERMALLY-ACCELERATED AGEING AND FTIR SPECTROSCOPY

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Introduction
Biodegradable polymeric scaffolds face a growing use in tissue engineering. However, changes in material properties during degradation can impact drastically the scaffold durability and therefore the efficiency of tissue reconstruction. Few studies focus on approaches allowing the prediction of the scaffold lifetime, while there is a need for strategies using accelerated testing protocols and versatile tools to easily investigate on the material degradation rate. In the present study, we investigated the thermally-accelerated ageing and lifetime prediction in culture medium of cross-linked poly(ester-urethane-urea) (PEUU) scaffolds [1].

Methodology
Elastomeric cross-linked poly(ester-urethane-urea) (PEUU) scaffolds have been developed through an emulsion technique allowing to produce highly interconnected porous structure [2]. Thermally-accelerated ageing was performed in cell culture medium at different temperatures: 37°C, 55°C, 75°C and 90°C. The degradation process was followed by gravimetry, swelling measurements, compression tests and Fourier-Transform infrared spectroscopy (FTIR). Compressive set measurements were also used as an indicator of the scaffold lifetime at 90°C.

Results
The study revealed that the PEUU scaffold degradation was associated with the hydrolytic instability of ester groups. As expected, the scaffold chemical composition variation over degradation was temperature dependant since the absorbance intensity associated to the ester stretching vibrations decreased with rising incubation time and temperature. Therefore, FTIR spectroscopy was used as a quantitative indicator of the hydrolysis content. The dependence of ester group cleavage on time of incubation was determined for each degradation temperature by regression analysis and Arrhenius type extrapolation was used to estimate the activation energy of the hydrolytic degradation reaction (80.84 kJ mol−1).
In the present study, the compressive set was selected as the failure criterion from the point of view of the scaffold functionality. For elastomeric material, the compressive set should not equal or exceed a value of 25%. Since the compressive set measurements set the scaffold lifetime at 90°C around 11.6 days of incubation in the degradation medium, the scaffold lifetime at 37°C was estimated to 1131 days (3.1 years) using an acceleration factor f equal to 97.5 as derived from the activation energy value.

Conclusion
It is well known that it is difficult to correlate in vitro degradation with in vivo expectation since in vivo conditions are more complex and lead to variation of the scaffold lifetime. However, the approach developed in this study could be a convenient way to simply and straightforwardly screen the durability of scaffolds when performing experimental design aiming to tailor scaffold lifetime.

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keywords: scaffold, accelerated ageing, lifetime

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VORONOI DESIGN OF ADDITIVELY MANUFACTURED 3D-PRINTED PCL-HA SCAFFOLDS: COMPREHENSIVE IN VITRO AND IN VIVO CHARACTERIZATION

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Introduction:
Highly porous biodegradable scaffolds made of polycaprolactone (PCL) and ceramic designed as three-dimensional (3D) guiding structures with rectilinear filling to facilitate bone regeneration have successfully been translated from preclinical studies into clinics as part of a scaffold-guided bone tissue engineering (SGBTE) concept. However, advances in 3D printer technology now allow the fabrication of novel scaffold structures, such as those with Voronoi design, which are potentially better able to mimic natural bone properties than those printed with rectilinear infill. The 3D Voronoi tessellation is based on random discrete seed points used to create cells that form a highly porous network structure with high mechanical strength. To pave the way for successful implementation, further developments of the SGBTE concept, such as the use of novel Voronoi scaffold design, are based on the principles of rigorous in vitro and preclinical in vivo testing to evaluate biocompatibility, biomechanical stability and tissue integration capacity.

Methodology:
Tubular composite medical-grade PCL hydroxyapatite (HA; wt% 96:4) scaffolds (outer diameter 10 mm, inner diameter 4 mm, height 15 mm) with 3D Voronoi tessellation were 3D-printed using additive manufacturing (BellaSeno GmbH, Germany). Scaffold porosity was assessed using micro-CT scanning (µCT 50, Scanco Medical AG, Switzerland). To determine the Young's modulus, unconfined, uniaxial compression tests (2 kN load cell) were performed under simulated physiological conditions of 1% phosphate-buffered saline (PBS) solution (37°C) with strain rate of 0.1 mm/s (30 kN Instron 5567, Melbourne, Australia). In vitro hydrolytic degradation was assessed over time by performing scanning electron microscopy (SEM), mass loss, gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) at time points of 0 (baseline), 30, 60, 90, 120, 150, and 180 days. During the 180 days PCL-HA scaffolds were immersed in sterile 1% PBS (10 ml) in closed 15 ml tubes, to avoid evaporation, and maintained in incubator at 37°C. At each time point, samples were washed three times with deionized water and incubated in vacuum overnight at 37°C before assessment. Biocompatibility of subcutaneously implanted scaffolds loaded with freshly harvested sheep bone graft materials was assessed using an an ectopic bone formation model of athymic nude rats. Assessment methods for in vitro and in vivo characterization included histology, immunohistochemistry, SEM and histomorphometry.

Results:
Scaffold µCT assessment revealed high mean porosity of 72.8% (± 0.94) (n=8). Further, the Youngs modulus of scaffolds (n=5) was 11.4 MPa (± 1.1). The PCL-HA scaffolds (n=7) exhibited slow degradation behaviour over the 180-day assessment period as observed with SEM, mass loss calculations, and molecular weight changes as determined by GPC and crystallinity with

DSC. Immunohistochemistry, Goldner's trichrome staining and SEM analysis of specimens (n=8) collected from rats (n=2) eight weeks after implantation show integrative physiological response at the interface between scaffold and different types of bone graft without signs of inflammatory reaction.

Conclusion:
High porosity and favourable biomechanical properties, along with slow and predictable degradation was observed. Histological examination showed good biocompatibility with no adverse host tissue reactions, making PCL-HA scaffolds with Voronoi design a suitable candidate for use in SGBTE.

keywords: scaffold; biocompatibility; regeneration

94238143146

HARNESSING THE IMMUNOMODULATION POTENTIAL OF NANOCLAY – AN ANALYSIS OF MACROPHAGE RESPONSE

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Inflammation is a protective response to damaged tissue and foreign bodies, such as biomaterials, and is usually considered to be negative. More recently, however, the active anti-inflammatory, pro-regenerative role of various mediators and inflammatory cytokines from immune cells have become widely recognised. In particular, macrophages play an essential mediating role in modulating inflammation and thus macrophage phenotype and function has received considerable attention.

Nanoclay has attracted attention in the field of regenerative medicine due to the inherent osteogenic bioactivity and ability of nanoclay to interact with proteins [1,2]. However, to date, no studies have explored how macrophages respond to nanoclay in terms of immunomodulation potential.

In this study, we have evaluated macrophage responses to nanoclay particles. Mouse bone marrow-derived macrophages were isolated from balb/c male mice (4-8 weeks old) and were cultured with various concentrations of nanoclay particles (50, 100, 500, and 1000 μg/ml) for 1 and 3 days. Intracellular and extracellular macrophages were observed by transmission electron microscopy, and the localisation of nanoclay particles in the cells was confirmed by energy-dispersive X-ray spectroscopy (EDX). Macrophage phenotype was evaluated by flow cytometry, and the concentrations of pro-and anti-inflammatory cytokines in culture media were measured by enzyme-linked immunosorbent assay (ELISA). Furthermore, the expression levels of pro- and anti-inflammatory cytokine related genes were assessed by quantitative polymerase chain reaction.

Macrophages actively phagocytosed nanoclay particles, regardless of nanoclay concentration. The nanoclay particles were found in extracellular macrophages, near actin filaments and intracellularly, e.g. within phagosomes and lysosomes. In the presence of 100 μg/ml nanoclay particles, in cell culture media, the population of M1-like macrophages dramatically increased for 24 hours ($p<0.034$). However, after 3 days, the population of M1-like macrophages decreased, but the number of M2-like macrophages increased. Similarly, significantly higher levels of anti-inflammatory genes, interleukin (IL)-10 and transforming growth factor (TGF)-β1, were observed in macrophages cultured in 100 μg/ml nanoclay particles for 3 days ($p<0.017$, $p<0.032$). For Tumour Necrosis Factor (TNF)-α, a pro-inflammatory cytokine, macrophages cultured with nanoclay particles, regardless of their concentrations, showed a significantly lower gene expression level compared to the macrophage without nanoclay ($p<0.039$). The current studies demonstrate the potential of nanoclay to modulate the phenotype of macrophages in vitro. Nanoclays can promote the development of M2-like macrophages expressing enhanced levels of the inflammatory genes, IL-10 and TGF-β1 with important implications therein for reparative processes in tissue engineering.

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keywords: Nanoparticles, Nanoclay, macrophages, immunomodulation

73296356408

EFFECTS OF SUBTOXIC CONCENTRATIONS OF VARIOUS METAL IONS ON MESENCHYMAL STEM/STROMAL CELLS

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The main risk factors for aseptic loosening and implant failure are wear and corrosion of metal implants. Despite a passive oxygen layer forming on many implant materials, electrochemical reactions can occur [1]. Oxidation and reduction reactions take place, resulting in a constant exchange of electrons and ions between the metal and the surrounding fluid [2]. The release of metal ions in different oxidation states can be promoted by the acidic pH caused by specific cells [3]. This in turn may have direct effects on the surrounding tissue and its cells [4]. The cytotoxic effects of nickel, cobalt, and chromium are mainly manifested by apoptosis, necrosis, and inhibition of DNA repair mechanisms [2,5]. However, the various metal ions differ in their local and systemic effects [2,3]. It is known that some metallic corrosion products can induce an inflammatory response in the implant periphery [4]. In this context, signal transduction processes influenced by metallic corrosion products may have an impact on cellular differentiation and immune response. The aim of this study is to better evaluate the low-threshold, “adapted” cellular responses.

In the present study, we treated mesenchymal stem/stromal cells (MSC) in vitro with different metal ion concentrations (starting with 10 µM nickel(II) chloride, NiCl2; cobalt(II) chloride, CoCl2, and chromium(III) chloride, CrCL3) and subsequently analyzed cell number and metabolic activity. In addition, the influence of the non-toxic metal ion concentrations (10 and 100 µM) on energy metabolism (more specifically, mitochondrial activity and extracellular acidification) was investigated by the Seahorse Analyzer (Agilent).

When exposed to higher ion concentrations (100 µM and above), the number of cells was reduced over the 3-day period with no evidence of cell death. This decrease correlated with relative metabolic activity (determined by MTS conversion assay). Specific examination of energy metabolism showed a reduction in basal respiration upon treatment with the metal salts CoCl2 and NiCl2. The trivalent chromium salt had no effect on basal respiration. In contrast, extracellular acidification, indicating glycolytic energy metabolism, was shown to be increased by NiCl2 and CoCl2 in a concentration-dependent manner over the course of the 3-day treatment. Again, CrCl3 exposure had no marked effect.

These in vitro results demonstrate that metal ions, as potential corrosion products of metal implants, can have a significant effect on cells even at non-toxic concentrations. Therefore, in order to prevent or treat aseptic loosening, the complex mechanisms of corrosion-induced

biological reactions should be fully elucidated.

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keywords: metal ions, cell survival, energy metabolism, mitochondrial respiration, extracellular acidification

62825430249

INDUCED MESENCHYMAL STEM CELLS AS A SECRETOME SOURCE FOR CNS REGENERATIVE THERAPIES: SIMILAR SECRETORY PROFILE BUT DECREASED REPLICATIVE SENESCENCE COMPARED TO BONE MARROW MESENCHYMAL STEM CELLS

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Although the secretome of mesenchymal stem cells (MSCs) has a great potential to be used in CNS regenerative therapies, tissue derived MSCs are a limited source which often need to be surgically harvested and present intrinsic donor variability. Induced pluripotent stem cells (iPSCs) conversely, can be easily generated and highly expanded from accessible somatic cell sources and differentiated into mesenchymal stem cells (iMSCs), which have been described to present a rejuvenated phenotype. Here, we aim to compare the replicative senescence of bone marrow derived MSCs (BM-MSCs) and iMSCs under human platelet lysate (hPL) supplementation and address its impact on secretome composition. For that, we have compared the proliferation and replicative senescence of iMSCs and BM-MSCs on a long-term culture, by cumulative population doublings, expression of senescence associated β-Gal (SA-β-Gal), P16-INK4A, P53 and P21 gene expression and the increase in cell area (hallmarks of senescence). Finally, we compared BM-MSC and iMSC secretory profiles by a non-targeted mass spectrometry approach, evaluated the concentration of important neuroregulatory factors on the secretomes of early and late passage

cells from both populations with a membrane-based antibody array, and assessed how the differences seen in these factors could affect their immunomodulatory capability with a mouse mixed glial culture. We show that iMSCs and BM-MSCs under hPL supplementation maintain their MSC properties and that iMSCs displayed higher proliferation, were capable of a higher total number of duplications and presented a decreased percentage of SA-β-Gal positive cells, decreased P16-INK4A and P21 gene expression and decreased increase in cell area compared with BM-MSCs. Furthermore, iMSCs and BM-MSCs presented a very similar secretory profile, with only 2 out of 136 proteins being significantly different in concentration in the proteomic analysis. Finally, the secretomes of both cells presented important neuroregulatory factors, with both having an upregulation of IL-6 and IL-8 at late passages which corroborated with a decreased immunomodulatory capability. In conclusion, we show that iMSCs can be expanded in hPL and that they have a similar secretory profile to BM-MSCs, but present decreased replicative senescence, therefore being a promising and more standardizable alternative to produce large quantities of secretome as needed for clinical purposes.

keywords: Secretome; Induced pluripotent stem cell; central nervous system

S19
Biomimetic Approaches to Cardiovascular Regeneration: how and why?
Room: S4 A
(28 Jun 2022, 13:30 - 15:00)

Conveners:
Petra Mela; Elena De-Juan-Pardo

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BIOMIMETIC APPROACHES TO HEART VALVE ENGINEERING: READY TO TELL YOU HOW AND AT WORK TO TELL WHY.

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Bioinspired polymer processing, with focus on improved control over biomaterial structure-function, is a research strategy that can play a critical role in facilitating the translation of a biomedical device. In this work, we utilize the specific example of tissue engineered heart valves to demonstrate this notion.
Valvular heart disease is currently treated with mechanical valves, which benefit from longevity, but are burdened by chronic anticoagulation therapy, or with bioprosthetic valves, which have reduced thromboembolic risk, but limited durability. Tissue engineered heart valves (TEHV) have been proposed to resolve these issues by implanting scaffolds designed to be replaced by endogenous tissue growth, leaving autologous, functional leaflets. This approach would putatively eliminate the need for anticoagulation and avoid calcification. Human heart valve tissue structure-function is still inadequately characterized and, despite the progress in scaffold fabrication strategies and encouraging results in large animal models, control over engineered valve structure-function remains at best partial. Moreover, while the notion of bioinspired control of structure and function is recognized as a promising strategy to enhance TEHV performance, the approach and its potential impact remain relatively unexplored in vivo.
We face these challenges by introducing double component deposition (DCD), a polymer electrodeposition technique that employs multi-phase electrodes to dictate valve macro and microstructure and resultant function. Engineered valve in vitro characterization included: leaflet thickness, biaxial and bending properties, and quantitative structural analysis of scanning electron micrographs. Results demonstrated the capacity of the DCD method to simultaneously control scaffold macro-scale morphology, mechanics, and microstructure while producing fully assembled multi-leaflet valves composed of microscopic fibers. The efficacy of this technology was further assessed in vivo in an acute (24 hrs) porcine model with the evaluation of three different devices: stented pulmonary valve (n=5), stentless tricuspid valve (n=5), and stentless mitral valve (n=2). Processing variables for these scaffolds were set to duplicate native heart valve tissue structural properties.
More recently, bioinspired DCD processed scaffolds have been implanted in an ovine model of pulmonary valve replacement with time point 1 (n=4) and 3 months (n=4,). Two groups were compared: scaffold with physiological leaflet thickness (120 μm) and scaffolds with over physiological leaflet thickness (240 μm). Explants at 1 month have shown a substantially higher extracellular matrix (ECM) production for the physiological thickness group. While these results suggest a more favorable tissue remodeling outcome for the physiological group and support the biomimetic approach, the mechanism for these preliminary observations remains unknown and re-iterate the urgent need for in-vitro platform able to elucidate the complex ECM process of ECM formation in vivo.

keywords: Tissue engineering heart valves, structure-function, in vivo model

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MELT ELECTROWRITING FOR TUNING THE PROPERTIES OF IMPLANT SURFACES

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Introduction
There is a large number of synthetic polymers, which are generally suitable as implant materials due to their chemical, biological or mechanical properties. What many of them have in common, however, is the challenge of growing properly into the body, which poses design demands specifically to the implant's surface. Requirements that many conventional plastic processing methods cannot meet, especially in the small size range. Here, we leverage the fiber forming technology Melt Electrowriting to additively structure polymer surfaces.

Methodology
We developed a method to apply a structured fiber layer onto solid polymer surfaces via Melt Electrowriting and showed this for various polymers used in biomedical applications. The resulting surfaces were assessed via contact angle measurements and cell adhesion tests. SEM confirmed the accuracy of the surface structuring patterns.

Results
Fiber pattern and sizes characteristic of Melt Electrowriting were successfully obtained on top of solid polymer surfaces. The effect of the deposited fibers on the surface properties were shown for different biocompatible polymers. Specifically, contact angle measurements and cell adhesion experiments showed promising results for the application as tissue engineering scaffolds.

Conclusion(s)
This study shows a valuable approach to optimize implant surfaces via Melt Electrowriting.

keywords: Melt Electrowriting, Micropatterning, Surface Modification

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MELT-ELECTROWRITTEN HIGHLY TUNABLE ANISOTROPIC SCAFFOLDS FOR CARDIOVASCULAR TISSUE ENGINEERING

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Introduction
Melt Electrowriting (MEW) is an versatile electric-field assisted fiber forming technique that has convincingly shown its potential for tissue engineering scaffolds both in vitro and in vivo. The additive manufacturing principle of MEW offers unparalleled possibilities to create precisley defined fibrous 3D architectures. The potential of design freedom with MEW is still largely unexplored. Here we present strategies for the automated design and digital fabrication of highly tunable ansisotropic scaffolds for in situ tissue engineering.

Methodology
We developed a MATLAB G-code design suite to automatically generate toolpath commands for the MEW setup. Scaffolds were fabricated from medical grade polycaprolactone (PCL). Their architecture and fiber diameter were assessed by scanning electron microscopy (SEM). Mechanical properties were determined by tensile testing, ingrowth of human umbilical artery smooth muscle cells was verified after 1 and 7 days of culture via SEM and (immuno)histology. Finally, the design strategies were validated for tubular constructs.

Results
All MEW scaffolds closely matched the coded designs. Highly tunable architectures were obtained, with fiber orientation and pattern strongly affecting the mechanical properties and anisotropy. Progressing cell ingrowth was verified in vitro after 1 and 7 days. Tubular scaffolds were exploited to show their potential for cardiovascular tissue engineering applications.

Conclusion(s)
This work further expands the capabilities of MEW towards the rational design and digital fabrication of fibrous scaffolds with controlled architectures and corresponding mechanical properties.

keywords: Melt Electrowriting, Anisotropy, Porosity, Cardiovascular,

62825427755

LAYERED VASCULAR GRAFTS - MECHANICAL PROPERTIES AND HEMOCOMPATIBILITY

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Cardiovascular diseases are the major cause of death worldwide. The lack of autologous vessels that can be used in cardiovascular surgeries compel engineers to look for nowel solutions. The main assumption of vascular tissue engineering is to design and produce functional materials that replace damaged blood vessels and restore their proper functions. Tissue-engineered vascular grafts with diameters >6mm are available on the market. However, the design and manufacture of prostheses with diameters ≤6mm is still a challenge for scientists due to their low hemocompatibility and thrombogenicity.

In this study two types of cylindrical layered structures with internal diameters ≤6mm were produced by the solution blow spinning method. The prostheses differed in the morphology of the internal surface. The first type of prostheses was characterized by a nanofiber inner surface, and the second was characterized by a solid one with small fibrous areas. The mechanical properties of the manufactured dentures were tested and compared. Then the surfaces of the prostheses were coated with polydopamine and biomolecules such as amino acids, short peptide sequences or polysaccharides were attached. The influence of the morphology of the internal surface and the presence of biomolecules on the hemocompatibility of the structures was investigated.

SEM analysis of grafts cross-sections has shown that manipulation of the solution blow spinning process parameters allows for the production of layered structures with differentiated morphologies of layers. Designed prostheses show high flexibility (Young's modulus value of about 2.5MPa) and tensile strength (maximum load value of about 60N). Grafts produced of medical-grade polyurethanes do not cause hemolysis. Activation and adhesion of blood elements to the inner surface of the prosthesis depend on its morphology. Fewer platelets were observed on nano-fibrous surfaces than on microfibrous/compact surfaces. Modification of the surface of prostheses with biomolecules also reduced the number of attached platelets.

In conclusion, the solution blow spinning method allows the production of layered cylindrical structures with internal diameters ≤6mm and desired mechanical properties, while the surface morphology and attached biomolecules affect the number of attached and activated platelets.

This project was funded in part by National Science Centre, Poland, grant number: 2020/39/I/ST5/01131.

keywords: tissue-engineered vascular grafts, solution blow spinning, hemocompatibility, layered prostheses, mechanical properties

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MESO-SCALE PATTERNED COLLECTING TARGET TO INDUCE LOCAL ANISOTROPY AND CURVILINEAR FIBER ORIENTATION IN ELECTRO-DEPOSITED, MICRO-FIBER BASED MITRAL VALVE SCAFFOLDS

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Collagen fiber network architecture in the native heart valve leaflets is characterized by preferential orientation and curvilinear arrangement that allow adequate stress distribution and effective leaflet coaptation. Specifically for the mitral valve, collagen fibers are preferentially aligned towards the circumferential direction with a curvilinear arrangement that runs from the posteromedial to the anterolateral commissure1. Tuning the collecting target tangential velocity is a common strategy shared by several techniques such as melt-electrowriting2, jet-spinning3, and Double-Component-Deposition (DCD)4 to achieve physiologically relevant structural anisotropy and circumferential alignment in the belly region of the valve scaffold. Similarly, conical shape collecting targets have been previously presented to obtain curvilinear arrangement in valve scaffolds5. However, the two methodologies cannot be combined. Tangential velocities can only induce circumferentially aligned straight fibers along the 3D geometry of a scaffold, while using conical mandrels produces curvilinear arrangement, which is strictly limited to 2D, planar scaffolds. In addition, the high tangential velocities requested to achieve physiologically relevant anisotropy are generally associated with deposition artifacts in complex 3D scaffold geometries. While the DCD processing method we previously introduced4, utilizes a collecting target made of an electrically conductive and a non-conductive component, this target design enables to manipulate the electrical field at the macro-scale and allows to recapitulate valve anatomy and to dictate various microarchitectural parameters, including fiber diameter and pore size. Yet, the curvilinear arrangement of the fiber network could not be achieved. In this study, we further advance the notion of DCD by manipulating the electrical field of the collecting target with mesoscopic grooves designed to induce local anisotropy and fiber undulation. A micro-grooved cylindrical copper mandrel was used as collector. To evaluate the effects of the groove geometry on the fiber deposition, three variables were considered: width, depth, and frequency which were set as equal to 50, 100, and 150µm. A cylindrical smooth mandrel was utilized as control. A tangential velocity of 0.26m/s, which normally generate isotropic scaffold on flat surfaces, was used for all the fabrications. The spatial electric field distribution was simulated in COMSOL-Multiphysics®. Morphological and mechanical properties of fabricated PEUU scaffolds were characterized by scanning electron microscopy, and biaxial tensile test. The width resulted in being the most effective parameter in terms of its capacity to induce statistically significant levels of circumferential fiber alignment and mechanical anisotropy. This notion was transferred to a collecting target specifically designed to reproduce the three-dimensional anatomy of the mitral valve, demonstrating control over fiber alignment and posteromedial-anterolateral commissure curvilinear arrangement. The in-silico model simulations allowed to visualize the electrical field distribution produced by the groove pattern and elucidate the likely mechanism of fiber deposition associated with local anisotropy at the tissue scale and the curvilinear fiber network at the organ level scale. This seminal study introduces a novel approach to design collecting targets for electro-deposition to advance

biomimetics in HV engineering.

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2.Saidy, et al., Front.BEBT, 8:793(2020).
3.Capulli, et al., Biomaterials, 133:229-241(2017).
4.D'Amore, et al., Biomaterials, 150:25-37(2018).
5.Hobson, et al., JBMMR-Part A, 103.9:3101-3106(2015).

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keywords: Tissue Engineered Heart Valve (TEHV), Double Component Deposition (DCD), electro-deposition, fiber undulation and anisotropy, mitral valve.

20941801806

DEVELOPMENT OF A BIO-INSPIRED SCAFFOLD FOR SMALL Ø VASCULAR REGENERATION

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INTRODUCTION
Cardiovascular disease is one of the major causes of death worldwide [1]. Synthetic vascular grafts (SVG) and autograft vessels are the current treatment modalities but, are ineffective for vessels with a diameter lower than 6 mm due to compliance mismatch [2] and limited in both supply and anatomical variability, respectively. An alternative solution is via tissue engineered vascular grafts (TEVG) [2-3] which aim to match the mechanical and biological properties of native vessels [3].
Melt electrospinning writing (MEW) is a recently developed technique that allows for the layer by layer assembly of micron diameter fibres in highly organised architecture that can be tuned to mimic the collagen fibre orientations found in the native vessel wall [3]. Therefore, the aim of this project is the development of a VG able to overcome current limitations of compliance mismatch and poor endothelialisation causing clot formation.

METHODOLOGY
A custom-made MEW printer was used to direct the deposition of polymeric micron-scale fibres in a bioinspired direction. Different aspect ratios were investigated in planar conformation to better tune mechanical behaviour, cell alignment and matrix deposition, which was then translated into a tubular conformation, through the use of a rotating mandrel.
MEW bioinspired scaffolds were infiltrated with a lyophilised fibrinogen sponge functionalised with heparin to prevent clotting. This hybrid construct was further wrapped in an electrospun elastic PLCL sheath to seal the graft.
Scanning electron microscopy (SEM) was used to investigate morphological characteristics. Pore size, porosity and degradation rate of the fibrinogen was also assessed for different crosslinking agents. Ring tensile test was used to investigate the mechanical properties of the grafts and compare them to those of a native porcine tissue. Biological evaluation of cell behaviour and extra-cellular matrix (ECM) production were performed to identify the best aspect ratio. Hemocompatibility and endothelialisation assay were also performed to validate the use of this off-the-shelf VG.

RESULTS
Our data demonstrates a preferential alignment of cell as well as ECM deposition along the major diagonal. The presence of fibrinogen enhanced cell seeding efficiency and ECM production while not effecting alignment and orientation. Mechanical data reported a response that resembles the typical J-shape of native tissue. The addition of a highly elastic layer of electropsun PLCL allowed for a higher resistance in deformation and recovery, additionally, the permeability was improved. The successful implementation of heparin allowed for a reduction of platelet adhesion that combined with a non-haemolytic behaviour demonstrate the suitability for vascular system application.
Thus, this bio-hybrid multi-layered graft represents a novel off-the-shelf solution to overcome current limitations of TEVG.

CONCLUSIONS
We successfully tuned tubular scaffold architecture, demonstrating high control and versatility.

The proposed mimetic bio-hybrid scaffold was identified as the ideal candidate to recapitulate mechanical properties, anatomical fibre orientation and ECM deposition of native vessel. Moreover, implementation of heparin demonstrates it suitability in an environment in contact with blood.

References
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Comment: This work is co-funded by an industry partner. Therefore, specific experimental details cannot be disclosed.

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keywords: TEVG - TUBULAR MEW - PLCL - FIBRINOGEN - HEPARIN

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DEVELOPMENT OF AN ADVANCED TISSUE-ENGINEERING SYSTEM THROUGH NOVEL 3D PRINTING FABRICATION METHODS

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Introduction: Ischemic heart disease is a major cause of human death worldwide owing to the heart's minimal ability to repair following injury. Despite medical advances, current treatments are not able to regenerate the damaged heart tissue. Therefore, alternative strategies are being assayed to identify the proper strategy to induce heart regeneration. In this sense, cardiac tissue engineering aims at obtaining cardiac constructs that mimics the native myocardium. Although major advances have been achieved in this respect, the generation of functional human mature tissue with physiological myocardial architecture and function to native adult myocardium remains a major obstacle. To address this, we have generated a 3D printable design that recapitulates not only the physical myocardial milieu, but importantly, 3D myocyte alignment, which is key to generation of maximum contractile force generation and therefore, maximum therapeutic efficacy.

Methodology: The designs have been 3D printed using Melt Electro Writing, an advanced printing technology uniquely capable of reproducing the properties of the cardiac extracellular matrix. Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (hiPSC-CMs) and cardiac fibroblasts (CFs) were embedded in a hydrogel (Fibrin or GelMA) and combined with MEW-designs in a composite system. Different ratios of hiPSC-CMs and -CFs (100:0, 95:5, 90:10 and 80:20 for CM:CF), as well as scaffolds fiber thickness (0.20, 0.35 and 0.70 mm) were evaluated for optimal tissue formation. Cardiac engineered tissues were maintained in culture for up to 4 weeks and characterized by histology, staining, ultrastructure, RNAseq, metabolic

and electrophysiological analysis.

Results: Overall, contractile human cardiac minitissues can be generated using both fibrin or GelMA hydrogels reinforced with 3D MEW printed structures displaying cardiomyocytes well distributed throughout the scaffold. No statistical differences were found between fibrin-based tissues containing only cardiomyocytes compared with those containing fibroblasts, in terms of beat rate, metabolic activity, gene expression, conduction velocity and activation frequency. However, contractile capacity for fibrin-based engineered tissues was markedly superior. Additionally, a longer remodeling process was required for GelMA-based tissues, whereas fibrin ones displayed an earlier coordinated beating. Also, cell and gel detachment were observed in GelMA-based scaffolds, making necessary the addition of high number of fibroblasts (20%) for optimal tissue formation. Metabolic maturation and transcriptomics analysis also highlighted the differences elicited by the choice of hydrogel. In summary, fibrin-derived tissues exhibited improved biological, structural, and mechanical properties compared with GelMA-based constructs. In vitro findings suggested that the composite fibre-hydrogel system may be a more suitable option for tissue-engineered heart repair.

Conclusion: We are progressing towards the rational development of engineered human cardiac tissues by a precise assessment of the main components, mimicking the unique 3D organization of the native heart architecture. Our results highlight the relevance of the choice of ECM-mimic (hydrogel), and provide and in-depth characterization of their differential effects upon the biology of the resulting tissues.

Comment:

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keywords: human induced pluripotent stem cells, cardiac tissue engineering, biofabrication, 3D printing, human heart

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3D PRINTING AND MULTILAYERED ELECTROSPINNING - A NOVEL METHOD TO PRODUCE BIOMIMICKING HEART VALVES

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Introduction
Currently used prosthetic heart valves show multiple limitations, including a reduced ability to regenerate. In this study we developed a three-layered electrospun heart valve using a dual electrospinning setup with a special 3D printed collector. In this manner, not only the microscopic but also the macroscopic structure of native heart valves was imitated. Biocompatibility of the used material constitutes an important property for clinical application. Re-seeding with human cells could allow for a regenerative approach.

Methodology
A heart valve shaped template was designed in commercial computer aided design (CAD) software, subsequently 3D printed and used for dual electrospinning. The polymers polycaprolactone (PCL) and polyurethane (PU) were electrospun from opposite sides onto a rotating collector (voltage = 15kV; flow rate = 3 ml/h; rotation speed for aligned fibers = 1520 rpm and 38 rpm for unaligned fibers). In a multistep approach scaffolds consisting of different layers with aligned or unaligned fibers were fabricated. Quality, morphology and orientation of the fibers were evaluated with fluorescence and scanning electron microscopy (SEM). Percentual porosity was assessed with gravimetric measurement. Biomechanical properties were determined by uniaxial tensile tests. Pseudomonas cepacia lipase was used for PCL degradation. Evaluation of the biocompatibility was achieved by static seeding of aligned and unaligned scaffolds with human fibroblasts. Cellular behavior was analyzed with SEM, histological and immunofluorescence microscopy.

Results
By CAD and 3D printing, it was possible to create an individual electrospinning collector, which precisely reproduces the macroscopic shape of a native heart valve. Thus, three-dimensional heart valve leaflets could be fabricated by using the collector in a dual electrospinning setup. To recreate the three layers (fibrosa, spongiosa, ventricularis) of the native valve, fibers were aligned circumferentially, randomly and radially. Homogenous, highly aligned (angle between fibers = 5.79 ± 1.61°) and unaligned fibers (no correlation possible) could be fabricated. Aligned fibers showed significantly higher tensile strength along the fiber direction than against it (15.72 ± 4.66 N/mm^2 vs. 1.83 ± 0.67 N/mm^2; $p<0.001$). Unaligned layers had an overall tensile strength of 6.48 ± 2.3 N/mm^2. High percentual porosity (85.81 ± 1.59% for aligned and 83.49 ± 1.74% for unaligned fibers) in all layers of the scaffold could be demonstrated. Especially within the unaligned dual spun scaffolds the percentual porosity could be significantly enhanced (89.3 ± 2.95%; $p<0.001$) by dissolving the PCL using enzymatic degradation. A homogenous monolayer of adherent fibroblasts on the surface of the scaffolds was observed in SEM, histological and

immunofluorescence staining. Furthermore, evaluation with SEM showed the formation of fibrin nets. This confirmed the biocompatibility of the material and its appropriate surface for cellular adhesion.

Conclusion
We established the development process of a biocompatible three-layered composite heart valve that replicates the fiber morphology as well as the geometry of a native aortic valve. The dual electrospun material was successfully seeded with fibroblasts, making it suitable for a regenerative approach. This method allows for individualized heart valve replacement by adjusting inserts of the 3D printed collector using personalized data e.g., CT scans.

keywords: electrospinning, 3D printing, heart valve, multilayered, biodegradable

S20
Biomimetic in vitro models for bone regeneration and cancer pathologies
Room: S4 B
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Silvia Farè; Gabriela Graziani

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ENGINEERING 3D HUMAN MULTICELLULAR BONE MODELS AS ANTI-METASTASTIC DRUG SCREENING PLATFORMS

Matteo Moretti (Ente Ospedaliero Cantonale, Regenerative Medicine Technologies Lab, Bellinzona, Switzerland)

The bone is a complex and dynamic tissue, in which the equilibrium between bone deposition and resorption can be perturbed by various pathological conditions, including bone metastases. Against them, no effective therapy has been developed yet, and available treatments are primarily palliative, aiming at restoring bone homeostasis. To improve the process of anti-metastatic drug discovery, new pre-clinical models are required, since available in vivo and in vitro models are limited by species specific differences in tumor mechanisms and by an oversimplification of the bone environment, respectively. Furthermore, potential side effects can be neglected with available models, resulting into unexpected toxicity of candidate drugs in clinical trials. In this scenario, advanced 3D in vitro models could become relevant assets for research and pharma industry for the discovery of new drugs against bone tumors, overcoming limitations of current models. To this end, our work aims at developing complex 3D in vitro models of bone tissue, taking into account its heterogeneous composition, to be exploited for the test of anti-metastatic drugs. To this end, we firstly developed microfluidic devices and millimeter-scaled vascularized bone models based on osteoblasts, osteoclasts, vascular cells and mesenchymal stromal cells embedded in a 3D hydrogel loaded with hydroxyapatite nanoparticles. We demonstrated that the simultaneous presence of all cell types and of the mineral component increased the bone turnover, as compared to simpler culture conditions. Then we added immune cells and breast cancer metastatic cells, showing that tumor cells were able to colonize the bone microenvironment, particularly in the perivascular niche. Furthermore, we were able to investigate the behavior of immune cells, when in co-culture with vascular and cancer cells within a bone-like environment. To test the effects of anti-tumor drugs in our system, we added rapamycin and doxorubicin, two FDA-approved anti-tumor drugs, known to have side effects on the vascular compartment, demonstrating how tumor cell resistance to the drugs was increased by the presence of a bone microenvironment. Furthermore, we were able to show the antiangiogenic effects of the drugs, by monitoring the damage to the microvascular network in the model. Finally, we tested our 3D bone model also with cells deriving from Ewing sarcoma, a pediatric bone tumor, showing that they could proliferate in our mineralized bone model. Sarcoma cell viability was affected by the knockdown of relevant genes and by the addition of oxidative stress inducers, in accordance with results shown in mouse experiments. Overall, we showed that the screening of anti-metastatic drugs in an in vitro model recapitulating the complexity of bone environment allowed to better estimate the effects of potential drugs both on their intended target and on other components of the microenvironment as compared to simpler models.

keywords: 3d in vitro models, cancer, vascular networks, microphysiological system, biofabrication

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IN VITRO TESTING OF BONE BIOMATERIALS - OPPORTUNITIES AND CHALLENGES

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Development of novel bone biomaterials inevitably has a phase of in vitro testing for cytocompatibility and functional osteogenesis. This relies on defined conditions under which osteoblasts, or their precursors, are stimulated to drive a mature, matrix depositing osteoblast phenotype. Based on the in vitro results obtained, a selection is made to be further tested using in vivo studies. While this is the classically adopted route, the correlation between in vitro and in vivo results is not as strong as would be expected (1). This may not be surprising when considering the methods typically being used are decades old. This suggests there is room to optimize in vitro tests to obtain more translationally relevant data. Often overlooked is the desired route to new bone formation. Direct osteogenesis is favored when cells experience 2D environments, while embedding cells within a hydrogel leads to a 3D environment, that may be more favorable to indirect bone formation, or endochondral ossification. Which raises the question: should the culture media used in vitro be tailored to the exact bone forming mechanism desired? It has been suggested that 10 mM β-glycerophosphate typically used in osteogenesis medium can lead to spontaneous ectopic calcification, an artifact often observed when using cell free material. Classical dexamethasone containing osteogenic media also has the potential to drive adipogenesis. However, many studies typically only investigate markers associated with an osteogenic phenotype, potentially overlooking conflicting signals driving differentiation into other lineages. Taken together, this suggest that improvements are possible.

Therefore, studies testing novel biomaterials are increasing in complexity with the aim to develop more representative models and more accurately represent in vivo conditions (2). Bioreactors can be used to improve flow and nutrient exchange, and coculture of cells can highlight interactions that may occur naturally in vivo but are lacking in monoculture studies. Increasingly, single cell sequencing is being adopted to study the differentiation of cells over time, identifying new markers and even distinct osteogenic differentiation pathways. This is key as the current range of markers may not be broad enough to make accurate predictions. Whole bone explant cultures can be combined with materials to investigate aspects such as osseointegration. As the body of knowledge increases, it is time to rethink how materials are tested

Combining bioreactors, coculture systems and improved markers and assays offers the opportunity to improve the accuracy of the in vitro results and reduce subsequent animal use as a result. While this may take some time, the outcomes will be rewarding.

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keywords: osteogenesis, in vitro, donor variation, bioreactors

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IN VITRO BONE MARROW NICHE FOR METASTASIS ASSAY

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Introduction: Bone marrow is one of the most preferable sites for metastasis, but the complicated in vivo metastatic niches make it challenging to study cancer cell colonization. We took a novel approach to establish an in vitro complex bone marrow environment on a dynamic 3D culture system, Bone marrow (BM)-on-chip. With a specific focus on prostate cancer, we designed, fabricated, and performed an in vitro on-chip cell culture to compare the tumorigenic potential of PTEN-negative PC3 prostate cancer cells with or without intact hemidesmosomes (HD).

Methodology: The BM-on-chip is designed in a standardized 96-well microplate format with a lumen structure, yielding a high throughput platform that allows co-culture and real-time observation of the colonization process. The 3D microenvironment of the bone marrow niche is created by sequentially loading tdTomato-expressing MC3T3 osteoblasts and GFP-positive PC3 cells into collagen I-coated microchannels at days 0 and 7 respectively. The cells are supplemented by the bi-directional flow of medium through the microchannels. Using Leica SP8 Falcon confocal microscope in a live-cell chamber, the assessment of osteoblast and PC3 cells co-culture was done first on day 14, followed by the addition of 0.5 nM or 1 nM DTX into the culture medium. The next imaging was on day 21 to analyze the effect of DTX on cell survival. The areas of MC3T3 cells were compared by analyzing the image data using IMARIS x64 9.2.1 software.

Results: HD-deficient PC3 cells were generated by knocking out the expression of α6-integrin subunit (α6-KO) using CRISPR/Cas9-mediated gene editing. We found that PC3 cells attached on top of fibrillar-shaped osteoblast and formed relatively small foci while α6-KO cells formed much larger cell clusters that were tightly integrated into the osteoblastic structures. Interestingly, co-culture of PC3 cells with osteoblast caused the reduction of MC3T3 osteoblasts whereas this effect was not observed in co-cultures containing α6-KO cells. Next, the cells were treated with docetaxel (DTX) which is a drug commonly used in prostate cancer treatment. Comparative analysis revealed dose-dependent reduction of PC3 cells area and volume after 7 days of incubation with DTX. In contrast, PC3 α6-KO cells appeared relatively resistant to DTX treatment, possibly due to their tight integration into MC3T3 osteoblasts. These observations are in line with our mouse model and 2D cells analysis results showing increased metastasis and DTX-resistance of HD-deficient α6-KO PC3 cells.

Conclusion: Our data shows that the BM-on-chip model can be successfully used for functional analysis of osteoblasts-prostate cancer cells co-culture. It reveals that PC3 α6-KO cells readily colonize osteoblast niches where they show robust resistance to DTX treatment when compared with control PC3 cells.

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keywords: bone marrow-on-chip; bone metastasis; hemidesmosmes

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BRIDGING THE GAP BETWEEN THE IMMUNE RESPONSE AND MINERALIZATION DURING FRACTURE HEALING

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Intro: Our broader understanding of the immune system's role in determining the success of intrinsic repair mechanisms has led to increased research focus on immunomodulatory therapies1. These therapies could be particularly effective in abnormal fracture healing, where bone tissue engineering has thus far failed to provide safe and reliable clinical therapeutics. While current developments rely heavily on endpoint assessment of bone formation in pre-clinical studies, the effect on key preceding processes, including hematoma formation, and the immune response to fracture, are seldomly taken into account2.
Aim: The aim of this study was to engineer an innovative in vitro model that facilitates studying the link between the immune response and mineralization during fracture healing.
Methods: Collagen-hydroxyapatite (CHA) scaffolds were incubated with human whole blood, mimicking the fracture hematoma formed when the scaffold is implanted in vivo. The effect of blood-biomaterial interactions on the microarchitecture of the scaffold, as well as the production of inflammatory mediators (IL-1β, IL-4, IL-6, IL-8, IL-10, MCP1 and VEGF), was assessed. The response of human bone progenitor cells (HBCs) to the blood-biomaterial interactions was evaluated in terms of cell infiltration, proliferation, and mineralization in the scaffold.

Results & discussion: The interaction between blood and CHA scaffolds led to the infiltration of erythrocytes, monocytes, and platelets, and the formation of a fibrin network. The porous microarchitecture of the scaffold was maintained in the presence of blood, while its support for HBC infiltration was limited by the presence of blood. The scaffold stimulated a limited production of pro-inflammatory factors (IL-6 and IL-8) by blood cells. However, the blood-biomaterial interactions significantly upregulated the production of IL-6 and IL-8 by HBCs by a factor of 10. The production of pro-inflammatory factors was temporally regulated, peaking between day 1 and 5, and tapered off by day 28. While blood-biomaterial interactions had no impact on the proliferation of HBCs over 28 days, blood impacted their capacity to mineralize, with a 28% reduction in calcium quantification, and a 50% reduction in intracellular alkaline phosphatase activity. Taken together, these data indicate that a transient hematoma-like pro-inflammatory matrix can be recapitulated in vitro, which can then be used to assess the effect of blood-biomaterial interactions on downstream processes of fracture healing, including mineralization. On-going experiments are assessing the capacity of blood-biomaterial interactions to alter the drug release kinetics of rhBMP-7, a commonly used inducer of bone formation, while the effect of rhBMP-7 on fibrin network formation and its ability to steer the immune response will be evaluated. Ultimately, the capacity of blood-biomaterial interactions to modulate the osteoinductive effects of rhBMP-7 will be determined.

Conclusion: In this study, a unique platform to bridge the knowledge gap between the immune response and mineralization during fracture healing was engineered, while also taking into account blood-biomaterial interactions, representing a significant advancement over current in vitro models of the fracture hematoma.

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keywords: Mineralization, immune response, blood-biomaterial interactions, monocytes

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BIOGENIC AND BIOMIMETIC NANOCOATINGS FOR BONE MODELLING AND REGENERATION

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Bone is a complex composite material, so any scaffold or model designed to reconstruct its integrity or model its behaviour must have a high degree of complexity and fulfil several requirements, including high biocompatibility, suitable surface and mechanical properties, adequate architecture, tailored degradability. Obtaining a bone-mimetic composition is also crucial, because hydroxyapatite (HA), the mineral phase of bone, dictates the behaviour of both healthy and tumour cells.
Here, for bone regeneration and modelling purposes, biomimetic nanostructured coatings are designed, manufactured by Ionized Jet Deposition (IJD) and deposited onto 3D printed metallic and polymeric scaffolds.
Coatings are obtained by ablation of biogenic hydroxyapatite, derived from bovine bone.
Polymeric scaffolds are obtained by Fused Deposition Modelling of PLA filaments, while metallic scaffolds are obtained by selective laser sintering starting from Ti-6Al-4V powder.
Surface morphology (FEG-SEM, AFM), composition (GI-XRD, FT-IR, EDS) and stability profile in culture medium (immersion in alpha-MEM at pH 7.4 and FEG-SEM at 24h, 7 days and 14 days) are assessed, for the coatings. Then, coverage of the 3D printed coatings is optimized and the relevance of shadowing effects is evaluated.
Finally, the interactions between the coatings and cells (MSCs for bone regeneration and SAOS-2 for bone tumour modelling) is assessed, by studying their adhesion to the scaffolds, morphology, early proliferation and differentiation (for the MSCs).
Coatings have a submicrometric thickness, that can be selected by tuning deposition time, nanostructured surface morphology and biomimetic composition. Indeed, the nanostructured coatings are constituted by multi-doped carbonated hydroxyapatite (Na 0.28 ± 0.08, Mg 0.16 ± 0.01 wt%) and are constituted of nanoscale aggregates (diameter ~ 50 nm) grouping in clusters up to 2 microns diameter. A nominal thickness of 450 nm is selected. Upon exposure in medium, they progressively dissolve, but maintain stability for over 14 days. Upon deposition on the 3D printed scaffolds, nanostructured coatings grow on all the surface of the fibres without altering their shape or porosity and coat their entire surface with no shadowing effects.
In addition, they promote cells colonization of the whole scaffolds, different from controls, where cells tend to concentrate on the outer layers.
For bone regeneration, coatings dictate MSCs morphology and sustain early proliferation and differentiation towards an osteogenic lineage. For bone models, they permit optimal viability at early and late timepoints.

As a consequence, the developed coatings appear promising for applications in regenerative medicine and bone modelling.

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keywords: orthopaedics, bone models, bone regeneration, nanostructured coatings, additive manufacturing

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ALGORITHMIC ENGINEERING ENABLING ORGANOTYPICAL PRINT TEMPLATES AT SCALE

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A limitation, when it comes to 3D printed biomimetic structures with micrometer and sub-micrometer precision are computer aided design (CAD) programs. Existing CAD software is usually based on “manual” step-by-step design principles intended and suitable for subtractive and formative manufacturing methods rather than organic designs for additive manufacturing. The resulting structures can hence deviate strongly from their natural tissue counterparts and small design changes of complex objects usually result in time consuming workflows [1–4]. Alternatively, tissue imaging dataset derived designs have been used[3,5] to recapitulate native geometries accurately, but lack systematic variation and adjustment of individual design parameters[1].
Algorithmic design based on parametric and algorithmic modelling provides an alternative and allows to efficiently explore and optimize geometries based on a set of logical operations and user defined rules.[6–8] Algorithmic design algorithms can yield hierarchical branching patterns resembling those found in nature[4,8] and enable scalable design automation (e.g. scan to print).
To experimentally realize perfusable biomimetic microtissue, we designed an alveoli network using algorithmic design principles. This lead to a set of hollow alveoli surrounded by a capillary network (A). Both alveoli and capillaries can be contacted via distinct in- and outlets for cell seeding, medium perfusion and tidal ventilation. This algorithmic design approach allows for deliberate design permutations such as alveoli size, degree of vascularization and wall thickness (B). Using dip-in mode TPS printing,[9] the algorithmic design was printed both with acrylate-based resin and imaged with scanning electron microscopy (C, left), as well as with gelatin-based resin and imaged with two-photon fluorescence microscopy (C, right).

keywords: algorithmic engineering, biomimetic, rapid iterations, design at scale

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BIOFABRICATION OF TUMOR MODELS THAT MIMIC THE TUMOR MICROENVIRONMENT USING EXTRUSION BIOPRINTING

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Cancer, as a cause of death, is only surpassed by cardiovascular diseases. Thus, it is critical to achieve progress in its treatment and prevention. Given the complexity and heterogeneity of cancer, various therapeutic targets are being investigated, including components of the tumor milieu. The tumor microenvironment (TME) consists of several types of cells (vascular cells, tumor-associated fibroblasts, immune cells, mesenchymal stem cells, and adipocytes) embedded in extracellular matrix soaked by interstitial fluid rich in soluble factors secreted by cells [1]. Increasing evidence indicates that tumor progression depends on the interaction between the tumor and its microenvironment and that the effectiveness of anti-cancer therapies is modulated by changes in the TME [1-3]. Therefore, extensive research efforts are devoted to investigating the spatial organization of the native TME and to build in vitro models of the TME using three-dimensional (3D) bioprinting [4] and tissue-on-a-chip techniques [5].
In this work, we report the 3D bioprinting of avascular structures that recapitulate several features of the TME [6]. In our model, the tumor is represented by a hydrogel droplet uniformly loaded with breast cancer cells, whereas the microenvironment is modelled by rings of hydrogel loaded with peritumoral cells: tumor associated fibroblasts and peripheral blood mononuclear cells. The tumor cells used in our experiments came from a commercial cell line (SK-BR-3), while the peritumoral cells were obtained from breast cancer female patients in different carcinoma stages. The cells were embedded in CELLINK Universal Bioink at concentrations of 1 million cells per milliliter, and the tumor models were fabricated using extrusion bioprinters (INKREDIBLE and BIO X, CELLINK, Sweeden). For the optimization and precise control of the printing process, we developed in-house Python scripts able to generate the G-code instructions for the two bioprinters based on the geometries of the digital models. Our workflow was designed to permit the subsequent bioprinting of desired constructs on multiwell plates of different dimensions. After two weeks of in vitro culture, histological cryosections of the tumor models showed that the hydrogel used in this study was appropriate for sustaining cell growth and proliferation. When tumor models were implanted subcutaneously, in the dorsal region of CD1 Nu/Nu immunosuppressed mice, within 28 weeks in vivo they became vascularized and grew about 5 times in diameter. In conclusion, our work presents a reliable methodology for building models of the TME using extrusion bioprinting. Such models can be used for fundamental research or, if built from patient-derived cells, for testing the effectiveness of anti-cancer therapies, thereby contributing to personalized treatment plans.

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keywords: extrusion bioprinting, breast cancer models, tumor associated fibroblasts, peripheral blood mononuclear cells

S21+S44
Biophysical Therapies - External energy to push internal regeneration + Nano Magnetic platforms - an attractive opportunity for advancing TERM products to the clinic
Room: S4 B
(30 Jun 2022, 15:30 - 17:00)

Conveners:
Paul Slezak; Peter Dungel; Alicia El Haj;
Luminita Labusca

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LEVERAGING PHYSICAL LIMITATIONS TO EXPAND SHOCKWAVE THERAPY TO NOVEL INDICATIONS

Cyrill Slezak (Utah Valley University, Orem, United States)

Recent efforts are beginning to explore the application of extracorporeal shock wave therapy (ESWT) to near lung tissue and transcranial indications. This is driven by a desire to expand the well establish therapeutic and regenerative benefits to a new group of patients. Through mechanotransduction of the incoming pressure pulse, a cascade of biochemical responses is triggered within the cells. Simultaneously, there is a non-linear response in the physical bulk properties of the targeted structures. Either one of those processes may be both beneficial or detrimental for the treatment outcome. We present findings on the efficacy of expanding ESWT to these areas currently consider counterindications. Treating near lung or brain tissue using low energy shockwaves holds an unlocked regenerative potential but has to be seen in the light of potentially destructive tensile forces imparted on the tissue. Based on reference measurements, computational simulations, and in-vivo experiments we explore competing considerations in moving towards a clinical use of ESWT in the potential treatment of a wide range of new indications.

keywords: Shockwave, regenerative medicine, simulation

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ANTIMICROBIAL EFFECTS OF BLUE LIGHT AND RESISTANCE DEVELOPMENT

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Impaired wound healing and infections present a major concern for our health systems. Combined with the steady increase of antibiotic resistances and lack of new antibiotic drugs, there is a pressing need for alternative treatment options, such as antimicrobial light-based therapies. While the germicidal properties of UV light have already been used for a long time, its negative side effects, for example DNA damage, photoaging and carcinogenicity, limit the possibilities and potential fields of application. Alternatively, blue light with wavelengths between 400 – 500 nm has shown promising results regarding the inactivation of a variety of microorganisms such as bacteria, yeasts and fungi viruses with the additive potential to improve wound healing (1). While the underlying mechanisms of blue light therapy have not been completely resolved yet, it has been proposed, that intrinsic chromophores e.g. porphyrins or flavins, generate ROS upon irradiation which destroy lipids, proteins and nucleic acids.
The aim of this study was to investigate the effects of LED devices emitting “soft” blue light with wavelengths > 430nm (provided by Repuls, Vienna, Austria) on the inactivation of gram-negative as well as gram-positive bacterial strains. It was revealed that the susceptibility towards the treatment differed greatly, presumably associated with the gene recA. In most bacterial strains, the protein RecA is responsible for homologous recombination, DNA repair and induction of the SOS response. Therefore, we hypothesized that the inactivation of the protein would increase the susceptibility of resistant strains and enhance the antimicrobial effects of the treatment. Although the potentiation of the therapy via RecA inhibitors was only partially successful, the influence of recA was confirmed by rescuing susceptible bacterial strains with the insertion of a plasmid carrying the gene. However, further experiments are needed to uncover the mechanisms behind the tolerability of blue light therapy and the importance of the SOS response. Nonetheless, progress was made towards a better understanding of the antibacterial actions of blue light. The modulation of the bacterial SOS response would not only benefit aBL therapy, but also antibacterial drug treatments, which are still the standard protocol for the care of infected wounds. Creating synergistic effects by combining these therapies into one singular treatment would likely increase therapeutic efficacy and is therefore a promising strategy to overcome the dilemma of antibiotic resistance.

keywords: Antimicrobial blue light therapy, disinfection, infected wounds, resistance

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HUMAN MESENCHYMAL STEM CELLS AND NANOMAGNETIC MATERIALS FOR REGENERATIVE MEDICINE

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INTRODUCTION: Iron oxide based magnetic nanomaterial, magnetic nanoparticles (MNPs) and magnetic nanowires (NW) are versatile tools in biology and medicine. MNPs-mediated drug delivery is tested for regenerative medicine (RM) purposes as well as for tumour treatment and diagnostics. Stem cell mediated delivery represents a modality to target remote, metastatic tumors or regenerative sites for controlled drug delivery. We recently reported that emote controlled actuation of MNPs-loaded cells can deliver micro-mechanical stimulation for modulating mesenchymal stem cell differentiation potential. NW platforms can be used to deliver topographical cues as well as magnetomechanical stimulation to differentiating MSCs.

METHODS: Human adipose derived stem cells (ADSCs) and Wharton jelly derived MSCs (WJMSC) loaded with proprietary were tested for viability, proliferative capabilities, culture induced senescence (beta galactosidase assay) and magnetic properties. In vitro osteogenic adipogenic potential of ADSCs-MNP as well as chondrogenic potential of ADSC and WJMSC –MNP exposed to magnetic field (MF) was tested. ADSC cultured on NW substrates with or without MF exposure were tested for viability and differentiation potential to mesenchymal lineages.

RESULTS: ADSCs-MNP and WJMSC-MNP complexes retain cell viability and proliferative capabilities compared to non-loaded and become controllable within MF due to high iron content. MNP presence decrease stem cells culture induced senescence. ADSCs–MNP display increased osteogenic and decreased adipogenesis when exposed to alternating magnetic field in a time, modality of exposure and MF intensity manner. ADSC-MNP displayed increased chondrogenesis compared to WJMSCs further increased by MF exposure. NW substrate supports attachment and viability of ADSC. Osteogenic conversion of ADSC cultured on NW substrates was found to be increased compared to plastic culture dish.

DISCUSSION & CONCLUSIONS: ADSCs-MNP display increased osteogenic and decreased adipogenic potential under alternating MF dependently on exposure protocol. ADSC-MNP but not WJMSC display increased chondrogenesis in vitro, further increased by MF exposure. NW substrate can be used to enhance osteogenic potential of ADSC in vitro. While this findings need to be confirmed in vivo experiments, MNP loading and NW substrated can be used to design innovative modalities for engineering of implantable bone and cartilage.

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keywords: magnetic nanoparticle, magnetic nanowires, mesenchymal stem cells

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A SIMPLIFIED PROTOCOL FOR PREPARATION OF CELL BASED BIOLOGICAL SAMPLES FOR OBSERVING NANOMATERIAL SURFACE ADHERENCE USING SCANNING ELECTRON MICROSCOPY IMAGING

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INTRODUCTION
When assessing the interaction between nanomaterials and cells, an important step is to image the effects induced by the material to the cell membrane. In order to evaluate the results of the interaction process, scanning electron microscopy (SEM) is typically used. Commonly, an important step during the preparation of biological cell samples for SEM is represented by the critical point drying, which involves the replacement of the alcohol used for dehydration with an inert gas. This conventional drying method is not only hard to accomplish, but it can lead to sample destruction if specific parameters are not met. On the other hand, when assessing nanomaterials adhered to the cell membrane, the integrity of the cell is not necessarily important, so a little cell deflation doesn’t affect the intended purpose of the evaluation. Here, we describe a simple and more cost efficient method to prepare biological samples for SEM imaging that preserves cell integrity and can be used to describe nanomaterials interaction with cell surface.

METHODS
Cells were grown on sterilized silica chips, after which the evaluated nanomaterial was added to the cell culture media at least 24h for incubation. Afterwards, the samples were washed to eliminate non-adhered nanomaterials, fixed with glutaraldehyde and osmium tetroxide, and dehydrated with increasing concentrations of alcohol. The silica chips were then air dried in the biological safety hood and in vacuum, followed by a sputter coat film of 5 nm of gold. The samples were imaged with a scanning electron microscope.

RESULTS
We were able to obtain well preserved biological cell samples, both with and without nanomaterials adhered to the cell membrane surface. Nanomaterials such as magnetic nanoparticles and magnetic nanowires were easily traceable on cell surface. Furthermore, the nanomaterials were clearly observed in the images obtained, while the cell surface was not affected by the drying process applied. Although the samples obtained using our method were characterized by a slight deflation of the cells, the morphology of the cells is well preserved and the method is suitable for the evaluation of the interaction between nanomaterials and cell surfaces.

CONCLUSIONS
We have described a novel cost efficient and easy to perform method for processing biological

samples for SEM imaging that preserves cell morphology and can be used for analyzing nanoparticle and nanowires interaction with cell surface.

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keywords: magnetc particles, nanowires, SEM imaging

31412768808

MAGNETIC NANOCARPETS BASED NON-INVASIVE MODULATION OF MECHANOSENSITIVE ION-CHANNELS FOR ENHANCED OSTEOGENESIS

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Introduction
Mechanotransduction is a key process in many developmental, physiological and pathological processes in bone. Mechanosensitive ion channels such as Piezo1 and TREK1 are the bonafide mechanotransducers that are critical for various biological processes, plays a critical role in bone regeneration1, 2. We already reported that the Magnetic ion channel activation (MICA) technology could apply mechanical force directly to mechanosensitive ion channels on the cell surface targeted with antibody functionalised magnetic nanoparticles for stimulating mechanotransduction and downstream processes3. Recently there is an augmented interest has been aroused to exploit Graphene Oxide (GO) and its derivatives for various biomedical applications. The abundant oxygen-containing groups in GO provides an excellent platform for further modifications using functionalised antibodies to facilitate targeted binding4. Therefore, we have developed a potential nanoplatform using magnetic nanoparticles and GO (GOMNPs) for enhanced osteogenic differentiation through the MICA based non-invasive remote activation. GOMNPs targeting the mechanosensitive Piezo1 and TREK1 ion channels were developed and their osteogenic potential under MICA application is studied with osteoblast-like MG-63 cells.

Experimental details
Characterisation of synthesised GOMNPs was done using TEM, XRD, Raman spectroscopy and VSM. All the cell culture experiments were done on MG-63 human osteosarcoma cells using GOMNPs. Effect of MICA stimulation on osteogenic differentiation was studied with ALP activity, Alizarin red and PCR studies of GOMNPs functionalised with Piezo1 and TREK1 under MICA application for 1h daily for 7,14 and 21 days.

Results & Discussion
The biocompatible GOMNPs were prepared using a simple, versatile strategy. The size and morphology of the as-prepared magnetic nanoparticles and the GO were confirmed using TEM and the structural features of the GOMNPs were evaluated by X-ray diffraction XRD and Raman patterns. The XRD and Raman patterns indicating the peak positions and the relative intensities of MNPs, GO and the GOMNP nanocomposites are successfully obtained. The obtained results also suggested that the synthesised GOMNPs showed excellent biocompatibility with superparamagnetic behaviour. The enhanced ALP activity and Alizarin red staining data indicated the positive effect of MICA stimulation on MG63 cells towards osteogenic differentiation. MICA stimulation mediated osteogenic gene expression by quantitative real-time PCR also confirmed the differentiation of MG-63 cells at mRNA level and improved expression of a panel of osteogenic markers, Runx2, ALP and OCN were obtained upon 7th and 14th days under MICA application

Conclusion
The preliminary in vitro results demonstrated the ability of functionalised GOMNPs for osteogenic differentiation under MICA treatment. The studies also proved that the functionalised GOMNPs were able to successfully bind with the mechanosensitive TREK1

and Piezo1 ion channels and was able to enhance the osteogenesis through mechanotransduction via MICA.

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keywords: Magnetic Nanoparticles, Mechanosensitive ion-channels, Mechanotransduction, Piezo1 ion channels, Osteogenesis

83767226884

MODULATING MACROPHAGE PHENOTYPES VIA IMMUNE-SWITCH MAGNETIC NANOPARTICLES

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Introduction: Inflammation is a physiological process in healing however, persistent inflammation signals hold deleterious consequences to the tissue and contributes to the inhibition of regeneration. Resolving inflammation remains an unmet challenge with great impact in the management of chronic inflammatory disorders and for the treatment of tissue injuries. Interleukin 4 (IL4) is a well-known key regulator of macrophage function by stimulating M2 phenotype (1), which is associated with the resolution of inflammation and structural tissue healing, and to dampen macrophage responsiveness to inflammation via IL4-pSTAT6 pathway. Despite IL4 promise towards tissue regeneration, hurdles in IL4 associated to in vivo instability and diminished bioactivity, demand for alternative vehicles to efficiently deliver IL4 and modulate macrophage functions, fostering resolution of persistent inflammatory cues with coordinated action over inflammatory cascades. Magnetic technologies offer promising tools that would precisely deliver biomolecules and control their action via IL4-pSTAT6 pathway (2), while providing contactless control, local retention and real time traceability using conventional imaging techniques. Thus, we propose to use superparamagnetic iron oxide nanoparticles (SPION) as magnetic field-responsive carriers for IL4 presentation, to remotely control immunoregulation of macrophages to favor the M2 phenotype via IL4-pSTAT6 pathway.

Methodology: Commercially available superparamagnetic iron oxide nanoparticles (SPION) were conjugated to a M2 macrophage promoter (IL4) via carbodiimide chemistry (SPION-IL4). The system was characterized according to dimension, shape, and charge as well as for IL4 binding efficiency. THP1-derived macrophages were used to investigate viability and the expression of immune-modulatory molecules in the presence of SPION-IL4. Two time-points (1h or 24h) were investigated, and two SPION concentrations (30 or 100 μg/mL) studied to insight on the impact of IL4 to drive M2 macrophages. These outcomes were compared against exogenous IL4 (Exo IL4)-stimulated THP1 (control group). A magnetic field was provided by a magnefect device (nanoTherics Ltd, UK) (350 mT/ well) for magnetic guidance and IL4 presentation to macrophages.

Results: Magnetically guided SPION-IL4 were shown to contribute for immune strategies participating in M2 polarization via IL4-pSTAT6 pathway. After 24h, our results have shown the levels of pSTAT6 trended higher in THP1 cells treated with SPION-IL4 comparing to Exo IL4. Furthermore, SPION-IL4-treated macrophages showed increased expression of M2 genes: IL10 and ARG1, and of M2 related proteins: CCL2 and IL1Ra, in comparison to Exo IL4, highlighting the effectiveness and impact of SPION-IL4 driving M2 signals.

Conclusions: This work reports the contribution of SPION-IL4 in IL4 mediated actions, taking advantage of SPION-IL4 magnetic responsiveness to deliver IL4 to macrophages and to promote M2 switch with the participation of STAT6. These findings show that SPION-IL4 influence

pro-regenerative features in macrophages, and that SPIONs hold potential to be explored as a magnetically controlled system for targeted delivery of immunomodulatory triggers or combined with more sophisticated systems aiming at strategies for improved tissue healing.

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keywords: Inflammation, cell-instructive systems, magnetic actuation.

52354540386

MAGNETICALLY MIRNA-BASED GUIDANCE OF MACROPHAGE FUNCTIONS

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Introduction: The currently available treatments for inflammation often target the symptoms but not the causes, leading to an ineffective management of persistent inflammatory conditions. Due to the central role of macrophages (Mφ) in the inflammatory response, and overall in healing, innovative strategies to fine-tune the states and functionalities of Mφ may unveil the pathophysiology of chronic inflammation with great promise for a wide range of human afflictions.
MicroRNAs (miRNAs) are powerful materials to program cell responses, offering immune-regulatory possibilities for resolution and prevention of uncontrolled inflammation (1). Nevertheless, efficient and precise delivery systems are still a challenge for translatable RNA-based technologies. A major obstacle is to find carriers that overcome the RNA instability and enhance intracellular release.
Magnetically-assisted strategies hold potential to modulate cell and tissue responses combining contactless control and tissue penetration for tracking, local retention, and real time monitoring (2). However, superparamagnetic iron oxide nanoparticles (SPIONs) have been scarcely explored for targeted delivery and cell programming. Therefore, we theorized that loading miRNAs onto previously functionalized SPIONs to transport them into cells may overcome the instability of miRNAs. Specifically, our aim is to magnetically deliver and study miRNA molecules in the modulation of Mφ responses by suppressing a miRNA sequence (miR-155-5p), known to be overexpressed in inflammatory states, and consequently increasing anti-inflammatory mediators.

Methodology: Commercially available SPIONs were conjugated with polyethylenimine and miRNA (miR-155-5p) to form magnetically-responsive complexes via electrostatic complexation (hereafter referred as magnetoplexes). The system was characterized according to dimension, shape, and charge as well as for miRNA binding efficiency. Stationary (SMF) and pulsed-electromagnetic field (PEMF) using MagnefectNano and MagnetoTherapy devices, respectively, were investigated for internalization and delivery of the magnetoplexes via magnetofection. THP1 cells were primed to an inflammatory state (Mφ1) with lipopolysaccharide and interferon-γ (100 and 20 ng/ml, respectively). Mφ1 viability and the expression of immune-modulatory molecules were assessed in the presence of the magnetoplexes. Two time-points (1 and 4 days) were studied together with different miRNA cargos (0.05 or 0.15 μg) to determine the impact of miRNA in inflammatory mediators. The outcomes were compared against non-treated Mφ1-primed THP1 (Ctrl).

Results: The magnetoplexes were successfully produced with 76±2nm, and 26.8±0.5mV. Iron as low as 40ng in magnetoplexes was effective for miRNA loading. An improved cell uptake was observed in SMF-stimulated cells comparing to PEMF. For that reason, a 20-minute SMF stimulus was used throughout the experiment. Additionally, intracellular magnetoplexes were detected by confocal microscopy. Four days after magnetoplexes treatment, anti-inflammatory molecules

as ARG1 trended higher in Mφ1, independently of miRNA mass, comparing to Ctrl. These outcomes suggest that controlled delivery of the miRNA to Mφ1 via magnetoplexes enables precision functional pro-healing changes.

Conclusions: The work combines contactless with high precision control to reprogram Mφ1 profiles, whose outcomes will contribute to advanced targeted and guided macrophage communication favoring a pro-regenerative environment and contributing to improved healing outcomes.

Acknowledgements: NORTE-01-0145-FEDER-000021; ERC CoG MagTendon No.772817; EC Twinning project Achilles No.810850; FCT Doctoral Grant SFRD/BD/144816/2019.

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keywords: Inflammation, miRNA, SPIONs, targeted delivery.

S22
Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine
Room: S4 C
(28 Jun 2022, 15:30 - 17:00)

Conveners:
Yuto Takemoto; Bernd Rolauffs

94355102706

IMAGE-BASED LABEL-FREE ANALYSIS FOR QUANTITATIVE AND REAL-TIME UNDERSTANDING OF CELLULAR STATUS

Ryuji Kato (Nagoya University, Nagoya, Japan)

Image-based analysis of cells is a powerful modality to measure and record the high content information which reflects the cellular status during their culture. Although morphology has been known for a long to contain significant information to monitor the transitions of cellular status, their analysis has been limited to experience-based interpretations. However, by the recent rapid development of image processing and AI technologies, we now have tools and platforms to tackle for understanding the real-time cellular status in a more quantitative manner. However, image-based analysis has been extremely limited to analyzing "labeled-images," and rare applications have been challenged to measure and utilize the heterogeneity information of cells for predicting cellular quality.
Our group has been reporting the label-free morphology-based analysis approaches for developing enabling technology for cell quality monitoring and control for maximizing the efficiency and reproducibility of cellular research and manufacturing [1-3]. Practically, from the time-course microscopic images, our data processing extracts not only the morphological descriptors of individual cells, but also the populational transition information, and tags them to the experimentally obtained cell quality ground-truth data. The key point of this technology is to combine the right combination of imaging hardware, automation technology, image processing, and data processing for objective performance. It should be noted that such a deep neural network structure is not always the best solution for such AI-based quantitative analysis.
In this talk, we will show the practical successful examples to apply such image-based label-free analysis for cell quality maintenance applications in (1) mesenchymal stem cell allogenic cell bank establishment, (2) single-cell morphological analysis for detecting senescence in mesenchymal stem cells, (3) novel optical challenge for label-free evaluation of spheroids. Our results show a high potential of image-based morphological analysis to enhance the quantitative understanding of the status of cells and their culture conditions. We also discuss the limitations and technological difficulties of AI-based image analysis compared to other image-based achievements in other fields for sharing the key points to enable the image-based cell evaluation successful.

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keywords: label-free image analysis, morphology, quality by design, cell manufacturing

83871204866

BASICS OF CELLULAR AND SUBCELLULAR MECHANOBIOLOGY

Günther Schlunck (Eye Center - Freiburg University Medical Center, Freiburg, Germany)

We will discuss the influence of cell shape and mechanical load on cell adhesions, cytoskeletal structure, cell signaling and transcription as determinants of cell fate and function.

keywords: mechanotransduction, cell signaling, cell adhesion

20941884069

CHONDROCYTE PROLIFERATION IS INFLUENCED MORE BY F-ACTIN DENSITY AND THE MACROSCOPIC TISSUE DISEASE STATE THAN BY CELL SHAPE OR MICROPATTERN GEOMETRY

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Introduction: Chondrocytes beneath the joint surface display a distinct superficial chondrocyte spatial organization (SCSO), which is a marker of tissue ultrastructure and function that undergoes a proliferative remodeling in early osteoarthritis [1,2]. Cellular filamentous actin (F-actin) and cell volume, two proliferation cofactors, change with osteoarthritis and might contribute to proliferation in early osteoarthritis. We asked whether chondrocyte proliferation rate (PR) can be controlled by controlling cell shape, and whether cell shape, the macroscopic tissue disease state (MTDS), or cytoskeletal F-actin intensity, density, or distribution has the strongest impact on the PR. To answer this question, we established a quantitative biology approach, combining single cell analyses with partial least square (PLS) analyses and random forest (RF) classification and regression predictive modeling.

Methodology: Chondrocytes were isolated from two MTDSs, macroscopically intact (MIA) or osteoarthritic-lesional areas (MOA), and cultured on custom-designed micro-patterned adhesion sites (MPs) for 1 or 7 days. Fixed chondrocytes were stained with phalloidin iFluor488 and DAPI and imaged using intensity-calibrated fluorescent beads for calculating F-actin intensity per cell and F-actin density. Fiji was used for textural analyses to quantify F-actin distribution, cell shape, and PR. PLS and RF were used to identify the most relevant factors for controlling and predicting PR.

Results: The PR of chondrocytes varied on different MP designs from 0.25 to 8.37, demonstrating that MP geometry allows minimizing and maximizing chondrocyte proliferation. PLS revealed that the highest impact on PR (in descending order) had cell shape (day_7), cell shape (day_1), and MP shape. The PR correlated negatively with cell solidity and roundness (day_7) and positively with cell area, length and aspect ratio (day_7), cell length being the most crucial factor. Interestingly, the PR of MOA chondrocytes was always higher than that of MIA chondrocytes. Using circular and H-shaped MPs in two sizes, we characterized single cell F-actin content, density, and distribution over time, revealing that F-actin density on day 1 was largely determined by MP size but not geometry or MTDS, whereas at day 7 F-actin density was determined by MTDS but not by MP size, geometry, or cell shape. PLS revealed that F-actin density (day_7), MTDS, and F-actin amount (day_7) had the highest impact on the PR, whereas other factors were relatively unimportant for controlling PR. Using RF regression with cell shape and actin parameters as input, the MTDS was predicted with an accuracy of 84.27 %, confirming

the relation of these parameters to the PR of human chondrocytes.

Conclusion: The PR of chondrocytes is not only controlled by MP or cell shape, but to a greater extent by F-actin density and MTDS. In cell culture, cytoskeletal F-actin density was initially dependent on MP size but this was superseded over time by F-actin density being determined by MTDS. Thus, these data suggest that in situ proliferation leading to loss of SCSO in early osteoarthritis appears to be more controlled by MTDS-associated changes in F-actin than MTDS-associated changes in cell shape.

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keywords: Osteoarthritis, chondrocytes, micropatterns, cell shape, F-actin

20941851177

MORPHOLOGY-BASED DETECTION OF SENESCENCE IN EXPANDED MESENCHYMAL STEM CELLS

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The expansion culture of cells is an essential process for manufacturing cells for therapeutic use. However, it is also an activity with a huge dilemma. This is because for clinical cell therapy treatment, and also for preparing cells for establishing cell bank, it is strongly required to expand cell number by passage culture to prepare a sufficient number of cells for applications, however at the same time, it is known that such over passage culture critically damages cell quality, especially in human mesenchymal stem cells (MSCs), therefore there is always a risk of establishing cell bank with quality decayed cells. Such balancing of production efficiency and cell quality is a critical issue to produce high-quality MSCs, however, the decision of such balance has long been relying on human experiences. To maximize the efficiency of obtaining high-quality MSCs for various applications, a more practical but efficient and quantitative method to enable non-invasive continuous monitoring of MSC's condition has been expected.

Our group has been developing "morphology-based cell quality prediction method (= morphometry)" by combining the recent image processing technology together with machine learning techniques [1,2]. We here propose the high detection performance of such morphology-based cell quality prediction method applied to evaluate senescence status in the expanded mesenchymal stem cells. In this work, we have intentionally prepared the over-passaged mesenchymal stem cells and measured their morphological descriptor profiles from the time-course microscopic images and their total expression profile by RNA-seq. From the machine learning of passage numbers and their morphological profiles, especially their population heterogeneity information, we succeeded in clearly discriminating the "over-passaged MSCs" which lost their growth potency and differentiation status. Moreover, our expression profile analysis indicated some novel marker gene expression profiles to target for understanding the quality decay in over-passaged MSCs. We also developed a novel image-based single-cell cytometry analysis method, to profile the heterogeneous cell populations in expanded MSCs, and show that there are several morphological categories to be detected for understanding the senescence type in MSCs.

keywords: Morphology, mesenchymal stem cells, senescence

41883633849

USING A MACHINE LEARNING-SUPPORTED APPROACH FOR ASSESSING AND PREDICTING THE SUSCEPTIBILITY OF ARTICULAR CARTILAGE TO MECHANICAL TRAUMA-INDUCED CHANGES IN CELLULARITY

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INTRODUCTION: The diagnosis and potential prevention of early post-traumatic osteoarthritis (PTOA) remain shot topics in orthopedic research because PTOA is among the leading causes of worldwide disability. In clinical routine macroscopic tissue damage after trauma is being diagnosed using imaging methods such as MRI or x-ray. Besides macroscopic damage, post-traumatic tissue regeneration vs. progressive degeneration to full PTOA depends also on the extent of cell death and survival, which can currently not be assessed clinically. We used here a clinically applicable score, the superficial chondrocyte spatial organization (SCSO) as a surrogate marker for articular cartilage ultrastructure and nanoscale functionality. We asked whether (i) the SCSO determines cell survival after simulated injury and (ii) can be used to predict the extent of cell death and survival. The ability to quantitatively assess a given patient's cell population after trauma and even predict his / her susceptibility to increased post-traumatic cell death would help improving diagnostic capability.

METHODS: Discs from human OA articular cartilage (AC) explants were fluorescently-labeled with Calcein AM (Thermo Fischer), a cell viability indicator, and Propidium iodide (Sigma- Aldrich), a cell dearth indicator. Each disc's SCSO was classified as chondrocyte string, double string or cluster organizations. One group of discs was subjected to mechanical injury (50%/0.5 s/0 s). Injured and control discs were stained for cell viability and cellularity, single cell morphology (area, length, width, circularity, roundness, and solidity), and multiple quantitative SCSO (qSCSO) parameters (nearest neighbor cell-cell distance (NNDs), cell intensity, and measures of cell grouping) were calculated for each disc. Both the morphological and the qSCSO data of viable cells were then used for training a random forest regression model (RF, R^2: 0.955) to predict the extent of cell death and survival. Statistical analyses were performed with SigmaPlot ($\alpha<0.05$).

RESULTS: A significantly higher number of chondrocytes organized as strings and double strings survived injury than chondrocytes organized in cell clusters and, conversely, a significantly lower number of chondrocytes organized as strings and double strings died after injury ($p<0.001$), demonstrating an SCSO-dependent susceptibility to trauma-induced cell death and survival. Chondrocyte morphology and qSCSO parameters were significantly different between SCSO stages and between injured vs. control discs ($p<0.001$). The RF model excellently predicted the amount of injury-surviving cells after trauma (R^2: 0.946).

DISCUSSION & CONCLUSIONS: The extent of chondrocyte cell death after trauma depends on the SCSO, revealing SCSO-dependent trauma susceptibility. Furthermore, qSCSO and single cell morphology can be used as machine learning predictors to predict SCSO-dependent trauma susceptibility with excellent precision.

keywords: chondrocyte, SCSO, machine-learning, single cell morphology, spatial organization

52354515605

PREDICTION OF M1, M2A AND M2C MACROPHAGE PHENOTYPES AND THEIR IL-10 PRODUCTION POTENTIAL BASED ON SINGLE CELL MORPHOLOGY AND PROTEIN INTENSITY USING A NOVEL MACHINE-LEARNING BASED APPROACH

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Introduction: Macrophages are a heterogeneous population of cells. In response to microenvironmental cues macrophages shift their polarization state, alter their phenotype and adopt pro- or anti-inflammatory functions, promoting tissue inflammation or contributing to its resolution. Using a novel high throughput image-based single cell morphology and protein intensity machine learning approach, we aimed to distinguish macrophage M1, M2a vs. M2c subtypes and determine whether cell shape could predict a cell's IL-10 immunogenic profile. This would aid in developing methods for assessing a cell population's inflammation modulating potential using machine learning.

Methodology: Blood-derived human CD14+ monocytes were isolated and matured into macrophages using GM-CSF followed by GM-CSF/TNF-α/IFN-γ (M1 macrophages) or M-CSF followed by either M-CSF/IL-4 (M2a macrophages) or M-CSF/IL-10 (M2c macrophages). Cells were then actin stained with phalloidin and DAPI for quantification of cell area, length, width, aspect ratio, roundness, circularity, and solidity. Cells were additionally immunostained for anti-inflammatory markers IL-10 and CD163 and the pro-inflammatory marker CD80. The resulting phenotypes were confirmed via a cytokine ELISA. The image-derived single cell morphology descriptors were then used to train a machine learning algorithm to determine how accurately cell phenotype could be predicted. We then asked whether machine learning could be used to predict IL-10 production potential, quantified as IL-10 intensity per cell.

Results: A number of classification models were generated based on cell shape and immunostaining data. When only shape parameters were included, macrophage phenotype was determined with an accuracy of <50% when comparing all groups (M0, M1 control, M1, M2 control, M2a, and M2c). The accuracy increased to >80% when only assessing M1 vs. M2 macrophages. Incorporation of staining intensity and density measurements for CD163+CD80 and IL-10+CD80 improved the accuracies to 84% and 79% for M1, M2a and M2c classes, respectively. For distinguishing M2 subtypes (M2a and M2c), an accuracy of 91% was obtained with the addition of CD163+CD80 co-staining and 88% for IL-10+CD80 co-staining. Importantly, a random forest regression model was able to predict IL-10 intensity based on only cell shape data with R-squared metrics of >95% for M2c, M2a, and M1-M2a-M2c datasets. Macrophage phenotypes were confirmed by release of TNF-α by M1 and IL-10 by M2 (M2c > and M2a) macrophages into the culture supernatants.

Conclusion: The use of single cell morphology and phenotype marker expression data was able to reliably predict M1, M2a, and M2c phenotypes when applied to a machine learning model. This is the first study to use cell morphology data to accurately predict M1, M2a, and M2c macrophage phenotypes. Moreover, the incorporation of machine learning regression analysis showed, for the first time, that cell morphology is sufficient to predict IL-10 production by macrophages at the single cell level. A number of cell shape descriptors were strong indicators of IL-10 staining intensities, which point to a means of predicting IL-10 production and thereby relevant anti-inflammatory properties, based only on cell morphology. This may provide the foundation for a generalizable strategy for identifying functional subpopulations of cells and predicting the functional response of IL-10-producing cells.

keywords: macrophages, IL-10, cell morphology, machine learning

94238154728

PREDICTION OF MEDICAL DEVICE COATING PROPERTIES VIA MACHINE LEARNING

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Introduction
Layer-by-layer (LbL) coating is a method for surface modification based on the electrostatic interactions between two polyelectrolytes. LbL coatings are used for multiple biomedical applications, because natural polyelectrolytes presenting good biocompatibility can be used for LbL film build-up. It is possible to develop antibacterial surfaces, smart healing materials, and coatings for tissue engineering. Moreover, LbL coatings can be used for loading drugs or other bioactive molecules, which allows their local delivery. Even though the mechanisms of LbL film development are well-established, the empirical manner of polycation/polyanion selection is an impediment on rapid new coating development, while the current health crisis has shown the importance of accelerated development of biomedical solutions such as antiviral coatings.

Methodology
In this work, we hypothesize that using the current state of the art data science techniques, we can determine how different parameters affect coating thickness and predict the thickness of the new coatings. To do so, we used historical and generated data for predictive model development using machine learning, an approach which uses algorithms that improve upon training on large datasets and is able to find complex patterns, make predictions and decisions.

Results
Using literature data and newly generated experimental results, we first analyzed the relative impact of 23 coating parameters on the coating thickness. Next, a predictive model has been developed using aforementioned parameters and molecular descriptors of polymers from DeepChem library. Model performance was limited because of insufficient number of data points in the training set, due to the scarce availability of data in the literature.

Conclusion
We demonstrate, for the first time, utilization of machine learning for prediction of LbL coating properties (1). It can decrease the time necessary to obtain functional coating with desired properties, as well as decrease experimental costs and enable the fast first response to crisis situations (such as pandemics) where coatings can positively contribute. Besides coating thickness, which was selected as an output value in this study, machine learning approach can be potentially used to predict functional properties of multilayer coatings, e.g. biocompatibility, cell adhesive, antibacterial, antiviral or anti-inflammatory properties.

References
1. Gribova, V. et al., Sci. Rep. 11, 18702 (2021)

keywords: Layer-by-layer, Machine learning, Medical devices, Coatings, Predictive models

S23+S31+S32
Can we bioengineer tissues using artificial cells? + Extracellular vesicles – next generation tool for diagnostics and regenerative medicine + Extracellular vesicles for soft tissue repair
Room: S4 A
(29 Jun 2022, 15:30 - 17:00)

Conveners:
Anne Des Rieux; Barbara Łukomska; Catherine Le Visage; Ewa Zuba-Surma; Paula Vena

62903402107

ARTIFICIAL CELLS WITH COMMUNICATIVE FEATURES, TOWARD HYBRID ORGANOIDS

Jan Van Hest (Eindhoven University of Technology, Eindhoven, Netherlands)

Artificial cells have been topic of intensive investigation over the past years. Although in first instance they have been mainly developed as a platform to attain a better fundamental understanding how living cells operate, they have recently also been recognized as interesting structures in biomedical research. In this lecture I will discuss an artificial cell system based on complex coacervates as developed in our group. These are formed by the mixing of two oppositely charged amylose derivatives that spontaneously interact and phase separate into a polymer-rich and polymer-depleted water phase. The coacervate microdroplets that are in first instance created are prevented from coalescence and further growth by a stabilizing polymer membrane. Coacervates droplets are interesting cell mimics since their crowdedness shows similarities to the dense cytoplasm of living cells. Furthermore, they have the ability to effectively sequester cargo. The polymer membrane we have created not only provides stabilization, but also allows exchange of molecules with the outside environment. We have demonstrated that biocatalytic cascades can be efficiently executed within one artificial cell, as a result of the high accumulation (150 fold) of the enzymes in the coacervate core. Furthermore, we have also been able to achieve uptake of protein cargo using affinity tagging; by incorporation of a Ni-NTA moiety in the coacervate, His-tagged protein were taken up highly effectively. By enzymatic removal of the His tag the proteins could also be controllably released. We have extended this uptake and release behavior to a DNA mechanism, which now allows for reversible shuttling of proteins in and out of the artificial cell, and between artificial cells. We have also investigated the biocompatibility of our artificial cells when in contact with living cells. After appropriate purification procedures we could demonstrate that the artificial cells are well tolerated. These developments have now opened up the way to combine artificial cells with living cells in the formation of organoids, in which the artificial cells are used as sending units of growth factors with spatiotemporal control.

keywords: artificial cells, communication, organoids

52419501284

FIRST STEPS TOWARD BIOPRINTING ARTIFICIAL CELLS

Daniela Duarte Campos (Heidelberg University, Heidelberg, Germany)

Artificial cells are biomimetic systems used to study properties of biological cells and to explore new possible applications in place of biological cells. Tissue engineering aims at replacing or recovering damaged or diseased tissues, or reconstituting tissues in vitro for disease modelling and drug development. Artificial cells can be combined with modern biofabrication strategies like bioprinting to generate bottom-up 3D tissues and organs. The Duarte Campos Bioprinting Lab investigates biofabrication technologies and biomaterials suitable for tissue and organ engineering, and their impact on the structure and function of natural and synthetic living tissues. This lecture will show our major achievements toward engineering lab-grown tissues and in vitro models using biological cells, and report on our first steps on the path to bioprinting tissues with artificial cells.

keywords: bioprinting; tissue engineering; biological cells; artificial cells; hydrogels

62825469128

TENOCYTE CONDITIONED MEDIA AND ITS POTENTIAL APPLICATIONS FOR IMMUNOMODULATION.

Amy Byrne (Keele University, stoke on trent, United Kingdom), Tina Dale (Keele University, stoke on trent, United Kingdom)

Tendon injuries are the top reported musculoskeletal injuries with Achilles, patellar, and rotator cuff tendons as the most common types of tendon injuryy (Ackermann & Institutet, 2013). Tendon injuries are a common cause of disability and account for decreased productivity. Both chronic and acute injuries can result in long-term pain and disability(Pabón & Naqvi, 2021). Extracellular vesicles, specifically exosomes, treatment has proven to enhance regenerative strategy by improving ; biomechanical properties, accelerating tenocyte proliferation and migration, reducing inflammation, and facilitating the healing at tendon–bone interface(Ren et al., 2021).

A THP-1 cell line activation model used to quantify the effects of tenocyte conditioned media (CM) on the proliferation and morphology of the activated THP-1 cells. When THP-1 cells are activated, they polarize into macrophages and monocytes depending on environmental signals. Flow cytometry was used to accurately characterize specific macrophage phenotypes in the presence of tenocyte CM by using CD-markers (CD14,CD25,CD197,CD204 CD206 and HLADR) to understand if the modulation triggered inflammation(M1) or supressed inflamation (M2) phenotype Exosomes isolated from tenocyte CM cultured at 21% and 2% oxygen concentrations then filtered (0.22um) before differential centrifugation (100,000g 90mins), pellets were analyzed for protein concentration and particle size then added to a density gradient prepared with Optiprep solution and spun at 100,000g for 16 hours. Protein concentration was obtained for 12 fractions, iodixanol content(via UV vis 230nm) and particle size (zetasizer). Fractions 4-7 (1.08g-1.22g) were spun again for 2hrs at 120,000g to remove idoxoanol ,finally the presence of exosomes was confirmed via Scanning electron microscopy and zeta sizer particle size data.

Tenocyte CM obtained at 21% and 2% oxygen concentrations, have higher levels of inflammatory cytokines when compared with serum free DMEM. Cytokine levels obtained via quantitative cytokine array (eve technologies Canada). Significant differences observed in cytokine levels (IL-2, IL-6 and IL-8) with 21% CM displaying higher levels (28ug,22,20ug/ml) than 2% oxygen. (19,16,15ug/ml) .The most abundant cytokines were; IL-2 IL-6, IL8, TNFa in CM in both oxygen concentrations. Flow cytometry utilizing; CD197, HLADR and CD86 we were able to demonstrate that activated THP-1 cells cultured in tenocyte CM showed a phenotypic shift towards higher levels of M1 macrophages (inflammatory state), at 21%. In 2% tenocyte conditioned media a lower level of expression of all but CD25 when compared to 21%, pointing towards hypoxia inducing a suppression of M1 and M2 macrophages. However, 2% CM THP-1 cells demonstrated higher levels of dendritic cells when compared to 21% (established by CD25 expression. Exosome preparations confirmed a protein concentration of 120ug/ml after density gradient ultracentrifugation, we further characterized via zetasizer which confirmed particles from 30nm-150nm and SEM imaging to confirm the presence of exosomes (via morphology and size). Futhur characterisation using ; tetraspanins and CD markers. Exosome preparations will be added to the THP-1 activation model in order to observe how exosomes influence the macrophage phenotype. Tenocyte conditioned media is able to modulate the immune system and stimulate healing.

keywords: Key words: Exosomes, cytokines, tendon, small molecule ,regeneration, inflammation, extracellular vesicles, musculoskeletal, immunomodulation, ultracentrifugation, density gradient, , exosome isolation.

94238103084

MATRIX-BOUND NANOVESICLES AS SELECTIVE MODULATORS OF THE IMMUNE RESPONSE

Hector Capella-Monsonis (McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, United States), Raphael Crum (McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, United States), Stephen F Badylak (McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsbirgh, United States)

Introduction: Matrix bound nanovesicles (MBV) have recently been identified as an inherent component of the extracellular matrix (ECM) and possess the ability to mitigate the proinflammatory activation state of macrophages. While the "anti-inflammatory" properties of MBV have several potential clinical applications, it is unknown if there is an associated compromise of the broader immune system. Stated differently, the systemic effects of MBV, and more specifically the effects of MBV upon the adaptive immune system and the ability to mount a protective immune response to pathogens is unknown and has not been explored.
Objective: To investigate the effects of MBV at systemic level and upon the adaptative immune response to pathogens.

Methodology: Biodistribution of MBV was assessed by fluorescence tracking after systemic administration intraperitoneally (IP) and intravenously (IV) in mice. Antibody and blood work analysis were carried out to assess biosafety. MBV modulation of pro-inflammatory activation was assessed in vitro in macrophages and in vivo in a psoriasis model in mice.
To assess the effect of MBV on the immune humoral response to pathogenic infection, mice were vaccinated with the pneumococcal vaccine PneumoVax™23 at day 0. Then, for 5 weeks, MBV (1012/mouse) were injected IP weekly, while a weekly dose of methotrexate was used as immunosuppressor control. Anti-pneumococcal polysaccharide IgG and IgM antibody titers were measured at days 7 and 28. At week 5 mice were infected with Streptococcus pneumoniae and the survival of the animals was recorded over 2 weeks.

Results: Antibody levels and bloodwork in healthy animals treated with MBV showed no relevant fluctuations nor biosafety concerns, whereas biodistribution showed accumulation in depurating organs such as liver, kidneys and spleen. MBV downregulated inflammatory markers in pro-inflammatory macrophages in vitro and meliorated the symptoms of imiquimod-induced psoriasis in mice corresponding with a downregulation of the IL-17/IL-23 axis, which corroborated the anti-inflammatory properties of MBV.
Pneumococcal vaccine-immunized mice with and without MBV treatment presented similar anti-S. pneumoniae polysaccharide IgG and IgM titers at days 7 and 28, demonstrating that MBV do not compromise the adaptative immune response. Contrary, immunized mice treated with methotrexate showed lower IgG and IgM titers. The functionality of the humoral immune response was further confirmed with the higher survival in vaccinated mice with and without MBV treatment, conversely to unvaccinated and methotrexate treated animals. Interestingly, when infected with a lethal dose of S. pneumoniae (107 CFU/mouse), 50% of mice treated with MBV after vaccination presented complete recovery after 14 days, whereas the rest of the groups showed no survival after 2 days of infection. These results suggest a boosting effect on the adaptative immune response elicited by MBV.

Conclusion: The anti-inflammatory properties of MBV do not compromise the ability of the adaptative immune system to build up a response against pathogens. Moreover, preliminary results suggest that MBV could further enhance the humoral immune response.

keywords: Matrix bound nanovesicles, immunomodulation, adaptive immunity, inflammation regulation

20941808655

MATRIX BOUND NANOVESICLES AS AN IMMUNOMODULATORY THERAPY FOR RHEUMATOID ARTHRITIS

Raphael Crum (University of Pittsburgh, Pittsburgh, United States), Kelsey Hall (University of Pittsburgh, Pittsburgh, United States), Catalina Molina (University of Pittsburgh, Pittsburgh, United States), George Hussey (University of Pittsburgh, Pittsburgh, United States), Emma Graham (University of Pittsburgh, Pittsburgh, United States), Hongshuai Li (University of Iowa, Iowa City, United States), Stephen Badylak (University of Pittsburgh, Pittsburgh, United States)

Purpose/Objectives: Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation and destruction of synovial joints that affects approximately 7.5 million people worldwide. Disease pathology, while multifactorial in etiology, is driven by an imbalance in the ratio of pro-inflammatory vs. anti-inflammatory immune cells, especially macrophages. Modulation of macrophage phenotype, specifically an M1 to M2, pro- to anti-inflammatory transition, can be induced by biologic scaffold materials composed of extracellular matrix (ECM). The ECM-based immunomodulatory effect is thought to be mediated in part through recently identified matrix-bound nanovesicles (MBV) embedded within ECM. While it is known that an M1:M2 disequilibrium contributes to RA disease progression, there are no therapies available that specifically modulate macrophage phenotype to promote disease remission through an M2, anti-inflammatory phenotype. There is thus a clear unmet need for developing approaches to modulate rather than suppress the immune response for the treatment of autoimmune diseases such as RA. The evidence supporting ECM- and MBV-mediated immunomodulation of macrophage phenotype, combined with the clinical evidence of pro-inflammatory M1 macrophages as a key mediator of RA, provides the premise of the present research. Using the pristane-induced, pre-clinical rat model of RA, it was hypothesized that MBV would reduce inflammatory arthritis disease development, decrease synovial inflammatory cell infiltration, prevent adverse cartilage remodeling, modulate synovial and systemic macrophage populations from a pro-inflammatory M1 phenotype towards an anti-inflammatory M2 phenotype, and thus promote disease resolution.

Methodology: Isolated MBV were delivered via intravenous (i.v.) or peri-articular (p.a.) injection to rats with pristane-induced arthritis (PIA). The results of MBV administration were compared to those following intraperitoneal (i.p.) administration of methotrexate (MTX), the clinical standard of care, using disease scoring, microCT imaging, histopathology, multiplex cytokine analysis, and multi-parameter flow cytometry.

Results: Relative to the vehicle treated animals, i.p. MTX, i.v. MBV, and p.a. MBV reduced arthritis scores in both acute and chronic phases of pristane-induced arthritis, decreased synovial inflammation, decreased adverse joint remodeling, and reduced the ratio of synovial and splenic pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages ($p<.05$). Both p.a. and i.v. MBV, but not MTX, reduced the serum concentration of RA and PIA biomarkers CXCL10 and MCP-3 in the acute and chronic phases of disease ($p<.05$). Flow cytometry dimensional data reduction with Uniform Manifold Approximation and Projection (UMAP) revealed the presence of a systemic CD43hi/His48lo/CD206+, immunoregulatory monocyte population unique to p.a. and i.v. MBV treatment associated with disease resolution.

Conclusion/Significance: The results show that the therapeutic efficacy of both systemic and local administration of MBV is equal to that of MTX for the management of acute and

chronic, pristane-induced arthritis, and further, this effect is associated with modulation, not suppression, of local synovial macrophages and systemic myeloid populations. The findings suggest that the immunomodulatory properties of ECM-based materials, specifically the MBV component of ECM-based materials, have therapeutic potential for diseases driven by a dysregulated immune system such as RA. The anti-inflammatory effects of ECM-based products have been well documented, and the expanded clinical applications made possible by MBV are worthy of further investigation.

keywords: Rheumatoid arthritis, immunomodulation, extracellular vesicle

20941802564

ELUCIDATING THE BIOGENESIS OF MATRIX-BOUND NANOVESICLES

Marley Dewey (University of Pittsburgh, Pittsburgh, United States), Stephen Badylak (University of Pittsburgh, Pittsburgh, United States)

Introduction. Matrix-bound nanovesicles (MBV) are nanometer-scale extracellular vesicles secreted by cells and found embedded within the extracellular matrix (ECM)[1]. MBV are similar to exosomes in size and shape, but MBV have distinctly different lipid profiles and RNA cargo[2]. MBV have demonstrated the ability to induce an M2-like pro-healing macrophage phenotype, promote neuronal stem cell differentiation, and suppress pro-inflammatory astrocyte signaling in optic nerve repair[3,4]. MBV have the potential to be used as a therapeutic and diagnostic tool, much like exosomes; however, an understanding of MBV biogenesis is lacking and hence their full potential depends on developing an understanding of the mechanisms of action. Our goal is to determine MBV biogenesis by answering the questions of the intracellular biogenesis of MBV, the mechanism of transport and binding to the ECM, and the mechanisms that govern their production.

Methods. To evaluate the intracellular origin of MBV, we used fluorescent lipid dyes to stain for lipids specific to cellular compartments. MBV and exosomes were isolated from the same cell source (fibroblasts) and were stained with lipids related to various cellular compartments (ie. Golgi) to determine if MBV or exosomes stained positively for these lipids and if we could live-cell image MBV production. To evaluate how MBV bind to the ECM we assess ECM-related surface markers on both MBV and exosomes and use high-resolution imaging to visualize vesicle location related to collagen production. Finally, we assessed the mechanisms that govern MBV production by sequentially deleting ESCRT-independent and -dependent subunits, a pathway in which exosomes use for their production, and assessing the impact of this on MBV production and collagen formation.

Results. We report a process for isolating MBV and exosomes from the same cell source, exosomes from the liquid phase and MBV from the deposited ECM. Ongoing work will be presented on the lipid staining differences between MBV and exosomes, and we expect that MBV and exosomes will have staining differences, as previous work demonstrates MBV have different lipid profiles from exosomes[2]. Ongoing data will be presented on ECM-binding surface proteins on MBV and exosomes, and whether MBV bind to collagen fibrils during or after collagen production. Finally, we show that GW4869, an ESCRT-independent pathway inhibitor, does not inhibit collagen and MBV production, possibly indicating that MBV do not use the ESCRT-independent process for production. We will further present the impact of ESCRT-dependent deletions on MBV and collagen production.

Conclusions. The present work will expand on our knowledge of a new class of extracellular vesicle, MBV. By understanding the mechanisms of their production, the origin of their production, and how they bind to the ECM, this will allow us to better realize their theranostic potential.

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[2] Hussey, G. S. et al., Sci Adv. 6, eaay4361 (2020).
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[5] Laulagnier, H. et al., Blood Cells Mol Dis, 35, 116-21 (2005).

keywords: Extracellular vesicles, matrix-bound nanovesicles, biogenesis, exosomes

83767236666

PLATELET-DERIVED EXTRACELLULAR VESICLES SHOW THERAPEUTIC EFFECTS ON A 3D TENDON DISEASE MODEL

Ana Luísa Ferreira da Graça (3B's Research Group, University of Minho, Guimarães, Portugal), Rui Domingues (3B's Research Group, University of Minho, Guimarães, Portugal), Isabel Calejo (3B's Research Group, University of Minho, Guimarães, Portugal), Manuel Goméz-florit (3B's Research Group, University of Minho, Guimarães, Portugal), Manuela Gomes (3B's Research Group, University of Minho, Guimarães, Portugal)

Introduction: Tendon diseases are common clinical problems that can dramatically affect the quality of life of individuals across the demographic spectrum. Current clinical approaches do not tackle the etiology of the disease, underlined by an unresolved inflammatory scenario that provokes hypercellularity, neovascularization, and a dysregulation of the critical balance between extracellular matrix (ECM) remodeling proteases and their inhibitors. In this regard, extracellular vesicles (EVs), a diverse group of nanosized membrane-enclosed particles actively released by all types of cells with key roles in communication, are being considered as very attractive therapeutic agents to trigger repair/regenerative processes in injured tissues. Thus, herein, the therapeutic potential of EVs derived from platelets was evaluated using a pre-establish 3D tendon disease in vitro model.
Methodology: First, bioengineered tendon disease models consisting of electrospun isotropic nanofibrous scaffolds coated with cell-laden hydrogels encapsulating human tendon-derived cells (hTDCs), were produced. Then, different platelet-derived EVs populations were isolated by differential centrifugation, added to hTDCs culture media, and their influence in cells phenotype and ECM remodeling was assessed over culture time.
Results: As expected, after 14 days of culture, a disease-like phenotype was observed in hTDCs of the miniaturized 3D tendon units. We verified that although EVs do not have a remarkable influence in hTDCs morphology, these are able to influence their biological response. Interestingly, the addition of EVs reestablish the expression of tendon-related markers like MKX, SCX, and TNMD in diseased hTDCs and decreasing the expression of osteogenic and fibrotic markers. Moreover, EVs increased the expression of different ECM components such as COL31A and DCN, and the expression of MMP-3, important factors in the balance between the synthesis and degradation of tendon ECM. Moreover, the presence of EVs was found to modulate the inflammatory response, as demonstrated by an increase of anti-inflammatory mediators, like IL-4, which might contribute to blunt the inflammatory processes occurring in damaged tissue.
Conclusions: Overall, we showed that platelet-derived EVs have a positive influence on tendon cells cultured on a disease-like in vitro model, not only by increasing the expression of healthy tendon cells markers and promoting ECM remodeling, but also by increasing the expression of anti-inflammatory cytokines. The beneficial effects of these vesicles are worthy to be explored in further studies to provide more insights on how EVs interact with tendon cells, becoming a promising therapeutic tool for tendon injuries recovery.

Acknowledgments: ERC CoG MagTendon grant agreement 772817; EC Twinning project Achilles 810850; FCT for PhD grant PD/59/2013 and PD/BD/135255/2017, Post-Doc grant SFRH/BPD/112459/2015, CEECIND/01375/2017 and 2020.03410.CEECIND.

keywords: extracellular vesicles; in vitro models; platelets; tendon-derived cells; tissue engineering

S24
Cell-rich constructs for tissue engineering
Room: S1
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Christina Schofield; Manuel Salmeron-Sanchez

41935606867

HIGH CELLS/BIOMATERIALS RATIO APPROACHES IN TISSUE ENGINEERING

João Mano (University of Aveiro, Aveiro, Portugal)

Bioinspired engineered microenvironments provide cells with a holistic “instructive niche” that offers the adequate entourage for cellular control both in space and time. Biomaterials provides essentially surface signals. We hypothesise that volume characteristics of biomaterials have a negligible influence on cell-behaviour and new tissue formation as compared with interfacial mechanical, topographic or biochemical properties. We explore this problem using minimalistic approaches, emplying scaffoldess strategies or by exposing cells to biomaterials that are designed to maximise the area to the volume. Examples from our group are presented, including: (i) a new concept of cell fiberoids, where fibres made of cells are engineered without any biomaterials; (ii) cell-sheets and cell-stamps obtained by magnetic force based-tissue engineering; (iii) cell encapsulation in liquified capsules with thin biomaterials shells for the autonomous development of microtissues; or (iv) ultrathin cell carriers with controlled geometry, and cell caps obtained by surface cell engineering. Such elements can be used as building blocks to be assemble into large constructs to produce macroscopic tissues using bottom-up tissue engineering methodologies.

keywords: bioinspired materials, engineering of cell microenvironments, multifunctional biomaterials

52354554306

PAPILLARY AND RETICULAR FIBROBLASTS GENERATE DISTINCT MICROENVIRONMENTS THAT DIFFERENTIALLY IMPACT ANGIOGENESIS

Adele Mauroux (Silab, Brive, France), Pauline Joncour (IGFL, Lyon, France), Noémis Brassard-Jollive (CIRB-Collège de France, Paris, France), Benjamin Gillet (IGFL, Lyon, France), Sandrine Hugues (IGFL, Lyon, France), Corinne Ardidie-Robouant (CIRB-Collège de France, Paris, France), Laetitia Marchand (Silab, Brive, France), Catherine Monnot (CIRB-Collège de France, Paris, France), Stéphane Germain (CIRB-Collège de France, Paris, France), Sylvie Bordes (Silab, Brive, France), Brigotte Closs (Silab, Brive, France), Ruggiero Florence (IGFL, Lyon, France), Laurent Muller (CIRB-Collège de France, Paris, France)

Vasculature plays an essential role in skin physiology and its architecture and function are altered in aged and diseased skin. Quite remarkably, papillary and reticular dermis show very distinct extracellular matrix (ECM) and vascularization. Furthermore, fibroblasts freshly isolated from their native microenvironment have different gene expression patterns, morphology and proliferation rate. Whereas 2D culture or embedding in hydrogels has been used to characterize both fibroblast subpopulations, the lack of relevant models has hindered investigation of their contribution to vascularization. We thus cultured human papillary and reticular fibroblasts as cell sheets over two to three weeks and used RNA-seq differential expression analysis to identify genes involved in microenvironment generation. Bioinformatics analysis revealed that cell sheet culture maintains specific expression of matrisome gene signatures resulting in papillary and reticular ECMs that differ in composition and structure. The transcriptomic also revealed layer-specific expression of angiogenesis-related genes. The impact of secreted and ECM-bound factors was then assessed using two independent 3D angiogenesis assays. The first assay consisted in stimulating endothelial cells embedded in a fibrin gel with conditioned media from each fibroblast subpopulation, while the second assay was based on cell-sheet co-culture of fibroblasts and endothelial cells, thus allowing direct interactions of endothelial cells with the microenvironment generated by fibroblasts. Vascularization was analysed in 3D using in-house developed software (3D-skel; Atlas et al, 2021) with both assays. These analyses revealed that papillary fibroblasts secrete highly angiogenic factors and produce a microenvironment characterised by ECM remodelling capacity and formation of dense small vessels, whereas reticular fibroblasts produced more structural components of the ECM associated with less but larger vessels. These features mimick the characteristics of both the ECM and vasculature of native dermis subcompartments. In addition to showing that skin fibroblast populations differentially regulate angiogenesis via both secreted and ECM factors, our work emphasizes the importance of papillary and reticular fibroblasts for tissue engineering and modelling dermis microenvironment and its vascularization.

Atlas Y, Gorin C, Novais A, Marchand MF, Chatzopoulou E, Lesieur J, Bascetin R, Binet-Moussy C, Sadoine J, Lesage M, Opsal-Vital S, Péault B, Monnot C, Poliard A, Girard P, Germain S, Chaussain C, Muller L. Microvascular maturation by mesenchymal stem cells in vitro improves blood perfusion in implanted tissue constructs. (2021). Biomaterials. 268: 520194

keywords: skin vascularization, cell sheet culture, microenvironment

62825462739

AN IN VITRO IMMUNOCOMPETENT HUMAN TISSUE-ENGINEERED MODEL OF ATOPIC DERMATITIS FOR DRUG TESTING

Inmaculada Barragan Vazquez (School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom), Simon Danby (Sheffield Dermatology Research, University of Sheffield, Sheffield, United Kingdom), Vicente Marco Garcia Gonzalez (Almirall S.A., Barcelona, Barcelona, Spain), Rosa Lopez Almagro (Almirall S.A., Barcelona, Barcelona, Spain), Maria Isabel Crespo Crespo (Almirall S.A., Barcelona, Barcelona, Spain), Helen Colley (School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom), Craig Murdoch (School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom)

Introduction: Atopic dermatitis (AD) is a chronic inflammatory and common skin disorder that is frequently associated with other atopic diseases such as allergic rhinitis and asthma. The immunopathogenesis of AD is a complex process, as both innate and adaptive immune systems are involved in the development of eczema in those patients. Keratinocytes, mast cells, dendritic cells, and T cells, among others, are involved in skin inflammation, however, in AD, there is a tendency towards T-helper 2 (Th2) responses. There is not a complete cure for AD, and most of the current treatments focus on symptom relief. Therefore, new treatments to improve the long-term control of AD are necessary. Murine models have been developed to mimic aspects of AD pathophysiology; however, they usually show markedly different responses to drug treatments that may lead to high failure rate of drug development.

Methodology: Purified peripheral blood human monocytes were differentiated into monocyte-derived dendritic cells (Mo-DC) using GM-CSF and IL-4. Purified naïve CD4+ T cells were CD3/CD28 activated, stimulated with IL-2 and polarised into Th2 cells using IL-4 and anti-IFNγ. Cells were characterised by qPCR and flow cytometry for key cell markers. To develop in vitro human skin models, type I collagen matrix populated with human dermal fibroblasts were seed on the apical surface with N/TERT immortalised human skin keratinocytes and Mo-DC, and cultured at an air-to-liquid interface before analysis. Th2 cells were incorporated into the dermal component.

Results: Mo-DC displayed successful differentiation by expression of cell-specific markers including CD1a, CD11c and CD207, and also high levels of proinflammatory markers such as IL-18 in response to allergens. Th2 cells showed increased CD119, D154, CD4 and CCR4, and secreted increased levels of IL-4, IL-5, IL-6, IL-13 and thymic stromal lymphopoietin (TSLP). Tissue-engineered skin models based on dermal fibroblasts and keratinocytes showed a keratinised, stratified squamous epidermis on top of a well-populated fibroblast containing dermis that histologically mimicked human skin. T cells were successfully incorporated into the dermis and Mo-DC into the epidermis, as determined by histological analysis.

Conclusion: Multiple immune cells can be differentiated from peripheral blood human monocytes and cultured in a 3D environment together with other human skin cells. The next aim is to show immune cell functionality within a 3D model in response to human allergens.

keywords: Tissue engineering, skin, immunology

62825458959

IS MORE ALWAYS BETTER? MODULATING HUMAN ADIPOSE DERIVED STROMAL CELLS CHONDROGENESIS TO ACHIEVE OPTIMAL BONE REMODELING IN VIVO.

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Introduction:
Stromal vascular fraction (SVF) cells, isolated from adipose tissue are an abundant easily accessible stromal cell source for bone tissue engineering. These characteristics make them a good alternative to bone marrow derived mesenchymal stem cells (BM-MSCs). Previous studies from our group provided a proof-of-concept that Adipose-derived Stromal Cells (ASCs), resulting from the expansion of SVF-cells, can generate bone tissue through endochondral ossification (ECO) by forming hypertrophic cartilage tissue (HCT) in vitro which in turn remodels into bone tissue when implanted in vivo. However, little is known about the underling mechanisms of ASCs chondrogenesis and their in vivo remodeling process.

In this work, we hypothesized that (i) freshly isolated SVF-cells are better suited for generating mature HCTs than expanded ASCs (P0-ASCs) and (ii) that more mature HCTs are more prone to remodel into bone tissue in vivo.
To that aim, we assessed the effect of SVF-cells monolayer expansion and its consequences on the proteomic signature and chondrogenic potential. In addition, we sought out to determine which HCT-properties can predictively determine the in vivo bone forming capacities of ASCs.

Methodology:
SVF-cells or P0-ASCs (0.5x106) were first seeded onto collagen sponges (V=40 mm3) and then maintained in chondrogenic cultures for 3, 4, 5 or 6 weeks. The cartilage maturation of these adipose-derived HCTs (A-HCTs) was evaluated qualitatively by histological staining and quantitively using Elisa and compared to the ones generated by BM-MSCs (BM-HCTs). Next, these HCTs were implanted ectopically in nude mice for 12 weeks to evaluate their bone forming capacities. In addition, the proteomic profiles of SVF-cells and P0-ASCs were compared by mass spectromer.

Results:
SVF-cells from all tested donors formed mature HCT within 3 weeks whereas P0-ASCs needed at least 4 to 5 weeks (depending on the donor). Longer in vitro differentiation increased the degree of maturation of HCTs which was characterized by a denser cartilagenous matrix and more mineralization. Interestingly, the HCTs degree of maturation obtained in vitro was indicative of their bone forming capacity in vivo, with an optimal bone remodeling obtained not for the highest degree of maturation but for an average degree of maturation. In fact, excessive in vitro maturation resulted in mineralized tissue rather than bone in vivo. In contrast, insufficient maturation resulted in major scaffold resorption in vivo. A-HCTs showed more mineralization, higher content of IL-10 and TNF-alpha relative to BM-HCTs. However, A-HCTs formed mature bone organ in vivo similarly to BM-HCT when suitably matured in vitro. When looking at their

proteomic profile, P0-ASCs presented a different profile to the ones of the SVF-cells; most notably regarding energy related pathways such as glycolysis, TCA-cycle and lipid metabolism.

Conclusion
Our data showed that SVF-cells possess a superior chondrogenic potential compared to P0-ASCs. In addition, we were able to not only control the degree of maturation of the HCTs but also modulate their in vivo fates.

Perspective:
We will assess whether a more physiological culture environment would better preserve the specific metabolic status of SVF-cells and thereby better retain their differentiation potential when expanded in vitro.

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keywords: SVF, Bone, Endochondral-Osiification, ASC

83767222599

LAMINARAN/PLATELET LYSATE-BASED HYDROGELS: TOO GOOD TO BE TRUE

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Our newly developed self-feeding hydrogels with enzyme-empowered degradation capacity have demonstrated high biological performance in-vitro and in-vivo as a novel self-maintained and biocompatible 3D scaffold1. Photo-crosslinkable platelet lysates (PL)-based hydrogels have exhibited to support distinct human-derived cell cultures owing to their high content of bioactive molecules, such as cytokines and growth factors2. To take advantage of all features of both PL and self-feeding hydrogels, here we combined UV responsive laminaran-methacrylate (MeLam) and PL-methacrylate (PLMA) derivatives plus glucoamylase (GA) to fabricate a multicomponent hybrid hydrogel (GLMPL). This hydrogel emerged as an unique scaffold due to the combination of sustained delivery of glucose produced via enzymatic degradation of laminaran and granting cell adhesin by presence of PL. Besides, this biomaterial was also applied to fabricate high-throughput freestanding microgels with controlled geometrically shapes. Impressively, such multicomponent hybrid hydrogel was successfully implemented as a glucose supplier bioink to fabricate complex and well-defined cell-laden structures using a support matrix.
MeLam and PLMA were synthesized following the previous reports2,3. In order to obtain a gradual production of glucose over time, GA enzyme was incorporated into MeLam/PLMA mixtures before UV exposure. We applied superhydrophobic surfaces (SH) patterned with wettable shaped domains (SL)4, where suspensions of cells and GLMPL hydrogel precursor could be dispersed and microgels with different geometries were produced after UV irradiation. To demonstrate the outstanding bifunctionality of such bioink, a mixture of GLMPL hydrogel precursor and cells suspension were deposited into agarose support matrix by extrusion 3D-printer. Thereafter the printed structures were exposed to UV to form gels. In vitro studies were performed on these biomaterials by encapsulating Human adipose-derived stem cells (hASCs) cultured in glucose free Dulbecco's Modified Eagle Medium. As such, any difference in cells response could then be attributed directly to the presence of enzyme and consequently glucose accessibility for the encapsulated cells.

CellTiter-Glo assay has shown hASCs metabolic activity significantly increased in GLMPL hydrogels over 21 days. Moreover, pronounced cell proliferation was confirmed through DNA quantification. Live-Dead assay confirmed encapsulated hASCs stretched inside the GLMPL hydrogel. DAPI/phalloidin staining have approved that cells elongated in GLMPL hydrogels and formed interconnected networks with neighbouring cells. Live-dead assay showed that up to 7 days of culture, most of the hASCs remained viable and elongated inside the microgels and 3D printed structures. It is noteworthy to mention that freestanding microgels and bioprinted scaffolds showed well-preserved architecture during the culture.
In conclusion, these results, combined that most current bioscaffolds suffer from lack of nutrient diffusion and adhere motifs, clearly suggest the potential of this multifunctional hybrid hydrogel in future developments of 3D structures in a wide range of biotechnological applications as an autonomous cell supporting system.

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keywords: Self-feeding hydrogels, Platelet lysate, Microgels, 3D-printing

73296312607

PERFUSION FLOW ON UROGENITAL EPITHELIAL CELLS FOR URETHRAL TISSUE ENGINEERING PURPOSES

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Background:
Urethral reconstruction is performed in patients with urethral strictures or for correction of congenital disorders. In most instances, foreskin or buccal mucosa flaps are used in these surgeries. However, complications may occur due to limited availability of tissue. In the future, tissue engineering (TE) might offer alternative solutions as it enables a detailed design to closely mimic native tissue, and provides opportunities for creating a scaffold for urethral reconstruction. Here we describe the cellular responses to fluidic flow of cell types that have the potential to be used in urethral TE.

Methods:
Human umbilical vein endothelial cells (HUVECs), and bladder and urethra derived epithelial cells (primary cells, isolated from male pigs by PdG) were exposed for 72 hours to fluidic, causing shear stress of τmax= 10.0 dyn/cm2 and τmax= 20.0 dyn/cm2 in a ibidi flow system. Cell elongation, cell alignment and actin fiber alignment were analyzed.

Results:
Bladder and urethra derived epithelial cells elongate and align when exposed to fluidic flow induced shear stress similar to endothelial HUVEC cells. Despite their different origin [1], we could see no differences between bladder and urethral epithelial cells in the flow experiments.

Conclusion:
Both bladder and urethral epithelial cells similarly adapt to fluidic flow. So in this respect, both cell types could potentially be used in TE for urethral reconstruction. This is important information, because the harvesting of bladder epithelium is much easier than of urethra epithelium: bladder epithelial cells can be isolated from urine or bladder washout or may be obtained by biopsy, in contrast to urethra epithelial cells. Next steps in our approach would be creating intermitted flow to mimic voiding patterns in patients

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keywords: Urology, Flow, Shear Stress, Cells

62825403004

INTERLEUKIN 1 BETA MODULATES THE EQUINE TENOCYTE TRANSCRIPTOME IN 3D CULTURE BY ENHANCING NF-KB SIGNALLING

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Introduction
Tendon injuries are common in equine athletes. Tissue healing occurs via biomechanically inferior scar tissue deposition, often resulting in re-injury. This is mediated, in part, by excessive proinflammatory cytokine release in the acute stages following injury. Stimulating equine tenocytes with interleukin 1 beta (IL-1β) alters tendon-associated and extracellular matrix remodelling gene expression in 2D culture and impairs 3D collagen gel contraction1. Similar findings were reported in humans tenocytes2, with activation of the NF-κB pathway suggested to facilitate these effects1,2. However, it is unknown how IL-1β modulates global gene expression in equine tenocytes in 3D culture and whether pharmacological NF-κB inhibition can attenuate the deleterious effects of IL-1β stimulation.

Methodology
Five biological replicates of adult tenocytes isolated from the equine superficial digital flexor tendon were cultured in a 3D collagen gel for 14 days under control or IL-1β-stimulated (1 nM) conditions, with media changes every 3-4 days. Daily gel contraction was examined before cells were harvested for RNA at day 14. Transcriptomic analysis was performed on a NovaSeq6000 platform, utilising Salmon-based alignment3 and differential expression analysis with DESeq2 (padj<0.05 and LogFC>1)4. Validation of several up-and-down regulated genes was undertaken with in-house qPCR. Gene Ontology and pathway analysis was performed with Panther Slim5 and Enrichr6, respectively. The interleukin 1 receptor antagonist (IL1Ra) protein1 and the NF-κB inhibitors JSH-237, IMD03548, and PF-066508339 were administered to tenocytes with-or-without IL-1β (1 nM); we are currently determining the rate of rescue for 3D collagen gel contraction, NF-κB P65 protein cytosol-nuclear shuttling after 60 min stimulation in 2D, gene expression after 72 hr in 2D, and IL-6 protein secretion with ELISA after 72 hr in 2D and during 14 days in 3D culture.

Results
Of the 18435 mapped genes, 2517 were differentially expressed following IL-1β exposure; 954 genes were upregulated and 1563 were downregulated. Gene ontology and pathway enrichment revealed that IL-1β enhanced NF-κB - but not JNK, P38, ERK, or STAT – signalling. Furthermore, IL-1β inhibited extracellular matrix organisation. IL1Ra fully rescued collagen gel contraction when co-administered with IL-1β, but only partially rescued tendon-associated and matrix remodelling gene expression at day 14. JSH-23 attenuated P65 nuclear translocation, reduced IL-6 secretion in 2D, and rescued matrix remodelling - but not tendon-associated - gene expression in 2D. Conversely, collagen gel contraction was not impacted by JSH-23 in the presence of IL-1β. Additional NF-κB inhibitor work is ongoing.
Conclusion

Exogenous IL-1β promotes NF-κB transcriptional signalling in equine tenocytes in 3D culture. These findings were verified with enhanced IL-6 secretion, a known target of P65. JSH-23 rescued IL-6 secretion and matrix remodelling gene expression after 72 hr in 2D, but had no influence

on collagen gel contraction during 14 days in 3D culture. As IL1Ra fully rescued collagen gel contraction, this suggests a more global attenuation of NF-κB signalling is required to restore tenocyte function as other NF-κB proteins (i.e., c-REL, P50) may target tendon contraction genes. This will be addressed with the ongoing IMD0354 and PF-06650833 experiments.

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keywords: Inflammation, Tendon, Horse, Cytokines

94238169579

OPTIMISATION OF BIOPROCESSING CONDITIONS FOR AN IMPLANTABLE MYOBLAST-MICROCARRIER COMBINATION FOR TREATMENT OF INCONTINENCE

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Introduction: Fecal incontinence has a high impact on patient quality of life. Available treatments based on surgical and non-surgical approaches range from change in diet, to bowel training or sacral nerve stimulation, none of which represent a long-term solution. Novel therapies are emerging that aim to regenerate the sphincter muscle and, therefore, restore continence. These approaches usually consist of administering a suspension of previously expanded cells to the damaged tissue. This strategy often results in a reduced cell viability due to the harsh step of cell harvesting from the culture platform in which cells were expanded, as well as the unnatural way the unattached adherent cells are delivered as a suspension.

Methodology: Here, we propose a new strategy for the treatment of fecal incontinence, by means of a two-step process. First, skeletal muscle cells (SkMCs) are expanded under static and planar culture conditions until relevant cell numbers are reached. The expanded SkMCs are then combined in bioreactors with implantable, biocompatible and biodegradable polymeric microcarriers, prepared by thermally induced phase separation (TIPS). Different bioreactor culture scenarios were tested: (1) SkMCs from different commercially available sources vs from primary muscle samples from different patients, (2) vertical wheel bioreactor (VWBR) vs spinner flask, (3) xeno(geneic)-free vs non-xeno-free conditions, (4) culture time, (5) agitation scheme. These parameters were optimized to maximize cell adhesion efficiency. Cell viability (calcein and DAPI staining) and distribution throughout the microcarriers, presence of the CD56 myogenic marker (flow cytometry), cell differentiation potential (desmin staining to assess myotube formation) and cell migration from the microcarriers were also assessed.

Results: The optimized adhesion process allowed us to obtain a 70-80% efficient SkMC adhesion onto the TIPS microcarriers. This was achieved by applying an intermittent agitation scheme to patient-derived SkMCs adhered to the microcarriers in VWBR, under xeno-free conditions, after 24h. SkMCs maintained their myogenic features (expression of CD56 marker) after the expansion phase in planar systems under static conditions, as well as after adhesion and culture in the microcarriers. SkMCs were able to migrate from the microcarriers and differentiate into multinucleated myotubes, as well as maintaining high cell viability throughout the process.

Conclusion(s): By optimizing the choice of bioreactor, as well as its operating conditions, we were able to obtain a high percentage of viable SkMCs adhered to TIPS microcarriers, with relevant muscle regeneration potential. Additionally, by performing the entire cell adhesion process under xeno-free conditions, we avoid the use of fetal bovine serum, which addresses regulatory issues. The xeno-free conditions established for the cell-microcarrier combination, associated to the single-use feature of the bioreactor selected, make this process more amenable to GMP compliance. The use of implantable microcarriers should also greatly increase the likelihood of success of the proposed cell-based therapy, as it avoids the drawbacks associated with harvesting of SkMCs and their subsequent delivery in an unattached state to the damaged tissue.

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keywords: bioreactor, CD56, fecal incontinence, SkMCs, TIPS microcarriers

S25+S64
Cellular senescence in tissue damage and regeneration + Understanding and preventing early inflammatory events that lead to development of osteoarthritis
Room: S4 C
(28 Jun 2022, 13:30 - 15:00)

Conveners:
Mikolaj Ogrodnik; Markus Schosserer; Melanie Hart

41935603186

CELLULAR SENESCENCE DURING AGING AND CHRONIC DISEASES: MECHANISMS AND THERAPEUTIC OPPORTUNITIES

Diana Jurk (Mayo Clinic, Rochester, United States)

Cellular senescence is characterized by an irreversible cell cycle arrest and a pro-inflammatory senescence-associated secretory phenotype (SASP), which is a major contributor to aging and age-related diseases. Clearance of senescent cells has been shown to improve brain function in mouse models of neurodegenerative diseases as well as obesity. However, it is still unknown whether senescent cell clearance alleviates cognitive dysfunction during the aging process. To investigate this, we first conducted single-nuclei and single-cell RNA-seq in the hippocampus from young and aged mice. We observed an age-dependent increase in p16Ink4a senescent cells, which was more pronounced in microglia and oligodendrocyte progenitor cells and characterized by a SASP. We then aged INK-ATTAC mice, in which p16Ink4a-positive senescent cells can be genetically eliminated upon treatment with the drug AP20187 and treated them either with AP20187 or with the senolytic cocktail Dasatinib and Quercetin. We observed that both strategies resulted in a decrease in p16Ink4a exclusively in the microglial population, resulting in reduced microglial activation and reduced expression of SASP factors. Importantly, both approaches significantly improved cognitive function in aged mice. Our data provide proof-of-concept for senolytic interventions' being a potential therapeutic avenue for alleviating age-associated cognitive impairment.

keywords: aging, senescence, neurodegeneration, senolytic

94238140408

CHARACTERIZATION OF CELLULAR SENESCENCE IN DEVELOPMENT, AGEING AND WOUNDING OF MOUSE SKIN BY CREATION AND EXPLORATION OF THE LARGEST SC-RNA-SEQ DATABASE OF MURINE SKIN CELLS

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Senescent cells, induced by various stressors, present a heterogenous population of irreversibly cell cycle arrested cells. They release pro-inflammatory compounds into surrounding tissue, collectively known as senescence associated secretory phenotype (SASP). Higher frequency of senescent cells is present during the developmental phase, regeneration and in aged organisms. Their accumulation during the ageing process increases risk and severity of age-related pathologies and is associated with formation of chronic wounds. Skin is a heterogenous organ and markers of senescence have been detected in several types of cells including keratinocytes, fibroblasts and melanocytes. As drugs targeting senescent cells show specificity towards selected pathways, that are known to be more active in some types of skin cells over others, it is of primary importance to decipher common and differential signatures of senescence in different populations of skin cells.
Here we present our preliminary in silico results on defining and characterizing phenotypes of senescent skin cells. We generated a database consisting of 19 studies with publicly available 10x genomics single-cell-RNA-sequencing (sc-RNA-seq) datasets of mouse skin from the developmental stage (embryonic), regeneration (wounded/unwounded) and the aging process (young, adult, old). To our knowledge this is the biggest sc-RNA seq dataset of mouse skin generated to date. Our dataset was created by harmonising and clustering readouts from all the studies (78 samples comprising 406.563 cells) in order to identify specific cell types. This dataset was used to unravel the phenotypic characteristics related to cellular senescence of different populations of mouse skin cells. Moreover, by splitting the dataset based on the presence of a known senescence marker we generated a list of gene ontology terms associated with senescent cells in skin development, homeostasis, wounding and ageing.

keywords: senescence, sc-RNA seq, big data, GO terms, senescence markers

41883640959

CELLULAR SENESCENCE IMPAIRS CHONDROGENIC DIFFERENTIATION OF MSCS VIA TGFB SIGNALING INTERFERENCE

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Mesenchymal stem/stomal cells (MSCs) are often studied for their possible tissue engineering applications. During in vitro expansion, however, MSCs enter a state of permanent growth arrest while remaining metabolically active; this phenomena is known as cellular senescence. Senescence can negatively affect tissue homeostasis and an increased number of senescence cells can be found in pathological tissues (e.g. osteoarthritic cartilage). Moreover, It has been shown that cellular senescence may alters the differentiation capacity of MSCs towards the osteogenic and adipogenic lineage, while little is known about the influence of cellular senescence on chondrogenesis. Therefore, the aim of this study was to determine the effect of senescence on chondrogenic differentiation capacity of MSCs.
Cellular senescence was induced in MSCs (N=4) either in monolayer prior chondrogenic differentiation, or at different time points during chondrogenic pellet culture (day-7 or day-14) using a 20 Gy gamma irradiation protocol. Senescence markers P16, P21 and IL16, and the β-galactosidase staining were used to confirm irradiation-induced senescence.
Chondrogenic differentiation capacity was induced by a standard TGFβ-based protocol for 21 days using a 3D pellet culture system, and evaluated by (immuno)histochemistry and RT-PCR.
To investigate the paracrine effect of senescent cells on recipient cells, we treated chondrogenic pellets using 2-day conditioned media from senescent cells and treat chondrogenic pellets for 24h, which were then analyzed for the expression of chondrogenic (SOX9, COL2A1 and AGCN) and catabolic (MMP-1, MMP-3, MMP-13 and ADAMTS4) markers. Western blot analysis on phosphorylated SMAD2 (P-Smad2 monoclonal antybody) was performed to identify TGFβ signaling activation. Non senescent cells or conditioned media from non-senescent cells was used as control.
When cellular senescence was induced prior differentiation, it abolished the chondrogenic capacity of MSCs with more than 95% reduction of GAG and Collagen type-2 deposition, as well as for all the chondrogenic markers measured by RT-PCR, in all the donor tested. A similar trend but with a less significant reduction was observed when senescence was induced at day-7 of differentiation. Interestingly, no effect on chondrogenic differentiation was detected when irradiation-induced senescent was applied at day-14 of differentiation. Moreover, medium conditioned by pellets cultures made of senescent cells had no significant effect on the expression of catabolic and anabolic markers measured by RT-PCR in recipient chondrogenic pellets. This suggests the negligible paracrine effect of senescent cells in our model.
In order to better understand how senescence was able to interfere with the chondrogenic process, we analyzed the ability of senescent MSCs to respond to TGFβ, the main pro-chondrogenic factor for MSCs. Upon stimulation with TGFβ1, phosphorylated-SMAD2 levels (an intracellular TGFβ effectors) were strongly reduced in senescent MSCs compared to control.

In this study we showed that cellular senescence reduced the chondrogenic differentiation capacity of MSC, but only when senescence occurs early during differentiation, and likely by negatively impacting the ability of the cells to respond to the pro-chondrogenic factor TGFβ1. This is a step forward in the understanding of the molecular mechanisms governing cellular

senescence in MSC, and towards better optimizing the use of MSC for tissue engineering applications.

keywords: cartilage, senescence, TGFβ, MSC, chondrogenesis

20941811955

A QUANTITATIVE TACK ON THE NANO CONSTRUCT FOR THE MODULATION OF INFLAMMATORY CYTOKINES IN BURN SCARS

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INTRODUCTION: Burn injuries propound copious challenges to clinical care and leaves the patient traumatized for years with scars. Even so, scarring walls off foreign bodies and seals injured tissue, it curb the movement and cede the cosmetic appearance of the skin. This wound healing defect is coupled with impaired cytokine expression. Upon tissue damage, the injured skin residents release cytokines into the wound bed to attract immune cells. Dysregulation in cytokine expression dramatically ends in defective wound matrix. Cytokines exerts inflammatory response to enhance wound healing. On the contrary, prolonged episodes of inflammation deposits excessive dermal matrix as scars. Thereupon, designing the construct to act concurrently by modulating the expressions of inflammatory cytokines is inevitable. An art of tuning the scar derma would be of a great value in post-traumatic wound healing. Thus far, clinically effective therapy for scar-less burns remains unmet and highly desirable goal. The fabricated nano-construct administered with biological agents exerts external stimuli to facilitate skin regeneration with minimal scarring.

METHODOLOGY: Polymers of oxyethylene and hydroxyalkanoates were electrospun with preloaded bioactive compound to obtain the multi-component nanofibrous matrix. The efficiency was investigated for the modulation in the expressions of pro- and anti-inflammatory cytokines specific to dermal scars in rat. The tissue lysates were taken from burned lesions of the rat and quantified for the cytokine expressions. A glass slide was spotted with targeted antibody for all inflammatory mediators. The sample diluents were diluted with the reference standard solution and the protein concentration matched tissue lysates. The experiment was performed and quantified with the median of the measured intensities to draw the final cytokine concentration (pg/ml).

RESULTS: In line, we have demonstrated that the anti-inflammatory cytokine IL-10 with the autocrine effect down-regulates the pro-inflammatory cytokine burst in the experimental group compared to the controls by displaying the diminished intensity of TNF-alpha, IL-1 beta, IL-6 and IL-8. The key growth factor TGFbeta-3 is at its peak which was contradicted to the level of TGF-beta-1 that aids in dense collagen turnover. The EGF was up-regulated with the activation of epidermal cells. The potent angiogenic factor VEGF, exhibited a moderate intensity in all treated groups.

CONCLUSION: Expanding knowledge in bio-materials and connective tissue research has resulted in the development of novel materials for variety of pathological conditions. The extremely complex phenomenon of burn wound healing involves a number of well orchestrated events. Burns attracts a high interest exploration in the field of medicine due to the physiological note of the skin. To date, none of the medicaments are as favorable as surgery in ameliorating the survival impacts in major burns. Here, the developed in-situ network of nano-construct aids physical guidance that exactly mimics the natural extracellular matrix for the guided tissue

regeneration with lesser possibility of excessive scars. This useful cost effective graft material pave the way to meet the requirements of the needy patients belong to the lower strata of the socio-economy where the expenditure towards is difficult to impossible.

REFERENCE: 1. Jovanovic J. et al., Glia 68, 574-588 (2020).

keywords: Wound healing, Biomaterial, cytokines, Scarring, Tissue engineering

41883623826

A COMPARTMENTALIZED JOINT-ON-CHIP MODEL TO UNRAVEL THE ROLE OF CARTILAGE AND SYNOVIUM IN OSTEARTHRITIS PATHOGENESIS

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INTRODUCTION
Osteoarthritis (OA) is the most prevalent degenerative joint disorder, but no reversing therapies are currently available [1]. This is mainly due to the disease complexity, that involves a failure of the entire joint, and to the disease multifactorial etiology [2]. Taking all this into account, a gap of knowledge still exists on initial disease mechanisms, linked to the unavailability of reliable human preclinical OA models [3]. In this scenario, organs-on-chip are promising candidates to deeply investigate tissues crosstalk in early OA stages. To this end, we developed a compartmentalized joint-on-chip model for the co-culture of cartilage and synovium, aiming at evaluating the disruption of the physiological cross-talk between these tissues that contributes to the pathogenesis of OA [4].

METHODOLOGY
The microfluidic platform consists of two separate culture areas, intended for synovium and cartilage 3D cultures, whose communication is controlled through valves that can be opened through vacuum application. An actuation layer allows to apply a mechanical compression to the cartilage compartment upon pressurization [5]. Human chondrocytes, and synovial fibroblasts and macrophages were separately cultured in the two compartments, respectively. To assess the effect of cartilage degeneration on triggering synovial inflammation, a hyperphysiological compression was first applied to the cartilage compartment to induce an OA phenotype [5]. Induction of synovial inflammation was then quantified upon valves opening through FACS analysis.

RESULTS
Culture conditions were first optimized to obtain mature synovium and cartilage microtissues separately, as demonstrated by deposition of extracellular matrix rich in Collagen type-I and lubricin, and Collagen type-II and Aggrecan, respectively. Then, an OA phenotype was successfully induced on cartilageupon application of a hyperphysiological compression, as proven by gene expression analysis, showing up-regulation of inflammatory markers (e.g. MMP13, IL8). Cartilage inflammation exerted a detrimental effect on synovial constructs, as the FACS analysis showed an increase in CD86+ and CD80+ macrophages upon valve aperture, indicating polarization towards M1 pro-inflammatory state.

CONCLUSIONS
The proposed compartmentalized microfluidic platform offers a solution to mature cartilage and synovial constructs and/or to induce OA traits in only one of the two compartments, by enabling a temporal control over chambers communications. The device was here exploited to prove that mechanically-damaged cartilage triggers inflammatory changes in the synovium, suggesting the possible role of cartilage as effector responsible for OA initiation. Furthermore, the platform is currently being used to elucidate the role of an inflamed synovium on cartilage degradation, and

this will eventually allow to understand which of the two tissues has a predominant role in early OA stages.

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ACKNOWLEDGEMENTS
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keywords: osteoarthritis, organs-on-chip, cartilage, synovium

73296332844

COMBINATION OF IL-1B AND IL-17A SYNERGISTICALLY INDUCE AN EARLY INFLAMMATORY AND DEGENERATIVE EXPRESSION PROFILE IN HEALTHY CHONDROCYTES AND SYNOVIAL FIBROBLASTS

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Introduction: IL-1β and IL-17 are highly present in the synovial fluid after joint trauma and are plausible factors in the development of post-traumatic osteoarthritis (PTOA). Although a growing number of studies have identified a role for IL-17 in OA, the mechanisms underlining the pathophysiological role of IL-17A in early PTOA disease in healthy joint cells remains unclear. We performed a side-by-side study comparing the effects of IL-1β, IL-17A and their combination in synovial fibroblast (SFs) vs. chondrocyte (CHs) in vitro cultures to understand the contributing role of each of these cells in mediating early inflammatory and degenerative effects in healthy cells of the joint.

Methodology: Healthy bovine CHs (passage 1 [P1]) and SFs (P1 containing macrophages and P5 consisting only of SFs) were stimulated with IL-1β, IL-17A and their combination for 3 days to mimic acute inflammation, then cultured in medium without or with cytokines for 3 additional days to understand the post-acute (PA) vs. persistent/chronic (C) inflammatory response, respectively. Absolute quantification of gene expression was performed by ddPCR at d3, d6(PA) and d6(C) for stimulated as well as unstimulated cells (CN).

Results: In healthy CHs and SF(P1) and SF(P5), IL-1β+IL-17A synergistically and significantly decreased COL2A1 (CHs) and increased IL-6 and IL-8 in almost all cell types after acute (d3) and persistent [d6(C)] inflammation. Three days after cytokine withdrawal, IL-17A led to post-acute gene modifying effects in all cell types and significantly increased IL-6 [in CHs, SFs(P1,P5)] and IL-8 [in SFs(P1,P5)] vs. CN, suggesting post-acute contributing effects of IL-17A in inducing inflammation within the joint. SF(P1) > SF(P5) ≥ CHs cultures expressed the highest levels of IL-6 (d3), IL-8 (d3 and d6C), and MMP-2 [(d3, d6(PA), d6(C)] in response to all cytokines, which may be due to the presence of macrophage-like synoviocytes. In response to all cytokine treatments and in all conditions tested [d3, d6(PA), d6(C)], MMP-3 expression tended to equally increase in CHs vs. CN. Moreover, MMP-2 expression significantly increased in response to IL-1β, IL-17A and their combination vs. CN [d6(C)] in CHs.

Conclusion: IL-17A and IL-1β synergistically induce an early inflammatory and degenerative expression profile in healthy CHs and SFs. While both cell types (CHs and SFs) contribute to the early events following treatment, IL-1β and IL-17A treated healthy SFs [(SF(P1)>SF(P5)] contribute more than CHs in upregulating the expression of inflammatory cytokines and the cartilage matrix-degrading enzyme MMP-2, which can participate in the degradation of a wide range of ECM proteins found in cartilage tissue and may be due to presence of macrophage-

like synoviocytes. This suggests that the combination of IL-17A and IL-1β synergistically induce early inflammatory and degenerative effects which could drive a chronic feedback loop of inflammation and degradation of cartilage tissue that involves cross-talk and feedback among chondrocytes, synovial fibroblasts and macrophage-like synoviocytes and thereby promote PTOA progression.

keywords: Osteoarthritis, IL-1β, IL-17

S26
Combined Korea-EU Symposium: "Bone from fat: Two distinct 17-18 year journeys in bone regeneration with adipose stromal/stem cells"
Room: S4 C
(30 Jun 2022, 13:30 - 15:00)

Conveners:
Gunil Im

62903403666

BONE FROM FAT: TWO DISTINCT 17-18 YEAR JOURNEYS IN BONE REGENERATION WITH ADIPOSE STROMAL/STEM CELLS

Gunil Im (Dongguk University BMC, Goyang, South Korea)

In this talk, author's decades-long research on inducing bone from adipose stem cell (ASCs) will be introduced, including 1) the exploration of osteogenic potentioal of ASCs versus bone marrow stem cell (BMSCs), 2) investigation of small molecules, peptides, gene transfer to enhance osteogesis, 3) synergistic effect of coculture of ASCs and BMSvs in osteogenesis and angiogenesis, 4) mining of de novo factors   that promote the survival and bone induction from poorly osteogegenic ASC clones.

keywords: osteogenesis, adipose stem cell, induction

94355104697

ADIPOSE-DERIVED CELLS FOR BONE REGENERATION: BONE (PRE)FABRICATION, DEVELOPMENTAL ENGINEERING AND VASCULARIZATION STRATEGIES

Arnaud Scherberich (Department of Biomedicine, University of Basel, Basel, Switzerland)

This lecture will show how biomaterials and components of the extracellular matrix, i.e. structural proteins and growth factors, affect the osteogenic potential of human adipose-derived mesenchymal stromal cells (ASC). Examples of bone formation by various human adipose derived cells-based engineered matrix/tissue, via either intramembranous or endochondral ossification will be presented. The lecture will also present the development of an advanced therapy medicinal product (ATMP) based on an intraoperative use of the stromal vascular fraction (SVF) of human adipose, containing mesenchymal and endothelial cells, to support bone repair with tissue harvest, cell isolation, seeding onto scaffolding material and implantation within 3-4 hours. A translation of this concept into a first-in-man clinical trial, demonstrating safety, feasibility and providing proof-of-principle of the biological functionality (i.e., bone formation) of the implanted graft will be presented. Another clinical case based on the use of such ATMP for mandibular bone regeneration will be shown. More recent and future research directions will be discussed.

keywords: adipose-derived cells, bone regeneration, vascularization, endochondral ossification, clinical trials

83767229706

INFLUENCE OF DEXAMETHASONE ON THE INTERACTION BETWEEN GLUCOCORTICOID RECEPTOR AND SOX9: A MOLECULAR DYNAMICS STUDY.

Filip Stojceski (Dalle Molle Institute for Artificial Intelligence (IDSIA-SUPSI-USI), Lugano, Switzerland), Gianvito Grasso (Dalle Molle Institute for Artificial Intelligence (IDSIA-SUPSI-USI), Lugano, Switzerland), Antoine Buetti-Dinh (University of Applied Sciences of Southern Switzerland (SUPSI), Bellinzona, Switzerland), Martin Stoddart (AO Research Institute Davos, Davos Platz, Switzerland), Elena Della Bella (AO Research Institute Davos, Davos Platz, Switzerland)

Objectives
The glucocorticoid receptor (GR) is a nuclear receptor that controls critical biological processes by regulating the transcription of specific genes. GR transcriptional activity is modulated by a series of ligand and coenzymes, where a ligand can act as an agonist or antagonist. GR agonists, such as the glucocorticoids dexamethasone (DEX) and prednisolone, are widely prescribed to patients with inflammatory and autoimmune diseases. DEX is also used to induce osteogenic differentiation in vitro. Recently, we highlighted that DEX induces changes in osteogenic differentiation of human mesenchymal stromal cells by inhibiting the transcription factor SRY-box transcription factor 9 (SOX9) and upregulating the peroxisome proliferator activated receptor γ (PPARG) [1]. SOX9 is fundamental in the control of chondrogenesis, but also in osteogenesis by acting as a dominant negative of RUNX2. There are still many processes to be clarified during cell fate determination, such as the interplay between the key transcription factors. The main objective pursued by this work is to shed light on the interaction between GR and SOX9 in presence and in absence of DEX at an atomic level of resolution using molecular dynamics (MD) simulations. The outcome of this work could help the understanding of possible molecular interactions between GR and SOX9 and their role on the determination of cell fate. Moreover, the impact of DEX on the previously mentioned macro molecular interactions will be fully clarified.

Methods
Classical MD has been used to perform a systematic investigation of a series of docked pose between GR, with and without DEX, and SOX9. HDOCK web server was used to obtain the initial docked configuration, considering as docking target for SOX9 the GR's dimerization and coenzyme moieties. All the SOX9-GR initial docked configuration were simulated in presence and in absence of DEX complexed with GR. The AMBER99SB-ILDN force field and TIP3P water molecules was used for defining system topology.

Results
The results showed that DEX has an influence on the binding behavior between SOX9 and GR. The SOX9 protein docked within the GR's coenzyme moiety showed a different binding behavior depending on the presence or absence of DEX bound to the GR's ligand binding domain. It is worth mentioning that when DEX is absent from the GR, SOX9 has the capability to strongly interact with the GR's coenzyme domain. Contrariwise, when DEX is bound, SOX9 has impaired capability to interact with the GR's coenzyme domain. Finally, no significant difference has been observed in the simulations of SOX9 docked the GR's dimerization domain, with and without DEX complexed to GR.

Conclusions
This work sheds light on the modulator effect carried out by DEX on the interaction between

GR and SOX9. The fruitful information extracted from this study may help the understanding at molecular level of the interaction between the nuclear receptor GR and SOX9 in the presence or absence of DEX, which can prompt a better understanding of the entire osteogenic differentiation pathway of human mesenchymal stromal cells.

[1] Della Bella E. et al. (2021) Int J Mol Sci 22 (9):4785.

keywords: osteogenic differentiation, mesenchymal stromal cells, molecular dynamics, protein docking

20941805655

NMR-BASED METABOLOMIC ANALYSIS OF ENDO- AND EXOMETABOLOME ADAPTATIONS THROUGHOUT OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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The use of mesenchymal stem cells (MSCs) for bone regeneration is a promising alternative to conventional bone grafts. Because local metabolic alterations seem to be critical for bone regeneration, metabolomics (through cell extracts and culture media) may unveil novel information on MSCs osteogenic differentiation,1,2 allowing their behaviour to be understood and potentially guided towards improved osteogenic lineage commitment (e.g. through specific media tailoring).3,4 However, only a few reports have monitored osteogenesis (mass spectrometry approaches predominating compared to nuclear magnetic resonance (NMR) spectroscopy), with scarce information interconecting intracellular and extracellular metabolic alterations.2,5 Here, NMR untargeted metabolomics is applied to monitor endo- and exometabolome adaptations of human adipose tissue-derived MSCs throughout 21 days of osteogenic differentiation. Endometabolome results revealed significant fluctuations in the metabolism of amino acids, energy-related compounds, lipids, nucleotides, and metabolic players in protective anti-oxidative mechanisms. Furthermore, exometabolome data highlighted alanine, glutamate, glycerol and citrate as important secretome components. Different metabolic stages are suggested, supported by putative biochemical explanations, and other important issues (such as inter-donor variability and aging effect) are discussed. Overall, this work has shown the great potential of NMR metabolomics to characterize the dynamic metabolism of MSC osteogenenic differentiation, ultimately enabling the potential discovery of universal biomarkers of osteogenic differentiation efficacy, with potential translation to in vivo clinical practice.

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keywords: Mesenchymal stem cells, Differentiation, Osteogenesis, NMR Metabolomics, Metabolism

52354509208

UNVEILING LIPID METABOLISM UNDERLYING AGING AND OSTEOGENESIS OF MESENCHYMAL STEM CELLS THROUGH 1H-NMR METABOLOMICS

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Mesenchymal stem cells (MSCs) are in the frontline of tissue engineering and regenerative medicine because they have indefinite self-renewal potential, and also exhibit great multilineage differentiation into a variety of tissues such as such as bone, cartilage, and adipose1. In the field of bone tissue engineering, the osteogenesis of MSCs is a promising therapeutic target, and controlling their differentiation is of critical importance for improving traumatic bone healing and therapy for genetic bone diseases2-4. Although metabolomics has already contributed to some extent understanding of the osteogenic mechanism of MSCs5, studies on the role of lipids metabolism are still scarce6.
In this study, we monitored the lipid profile of human adipose-derived MSCs as a function of osteogenic differentiation times, using nuclear magnetic resonance (NMR) untargeted lipidomics strategy applied to MSCs. Samples were collected at different time points during 2D culturing in both control (undifferentiated) and in osteogenesis-inductive media. Changes in the lipid composition of human adipose-derived MSCs were interpreted in detail based on multivariate and univariate statistical approaches. Our 1H-NMR strategy detected various groups of lipids with statistically relevant changes throughout the whole 21-day period of osteogenic differentiation. These comprised phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, plasmalogen, total fatty acids, polyunsaturated fatty acids and unsaturated fatty acids, as well as total, free and esterified cholesterol. These results may unveil potential osteogenesis-related lipid biomarkers, which are essential to provide a comprehensive picture of the MSCs differentiation metabolism.

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keywords: Osteogenesis, Mesenchymal stem cells, Lipid metabolism, NMR metabolomics, Lipidomics

41883658008

CONVERGENCE OF SCAFFOLD-GUIDED BONE REGENERATION PRINCIPLES AND MICROVASCULAR TISSUE TRANSFER SURGERY

Dietmar W. Hutmacher (Queensland University of Technology, Brisbane, Australia), David S. Sparks (Queensland University of Technology, Brisbane, Australia), Flavia Medeiros Savi (Queensland University of Technology, Brisbane, Australia), Constantin E. Dlaska (Queensland University of Technology, Brisbane, Australia), Siamak Saifzadeh (Queensland University of Technology, Brisbane, Australia), Gary Brierly (Queensland University of Technology, Brisbane, Australia), Edward Ren (Queensland University of Technology, Brisbane, Australia), Amaia Cipitria (Biodonostia Health Research Institute, San Sebastian, Spain), Johannes C. Reichert (Queensland University of Technology, Brisbane, Australia), Marie-luise Wille (Queensland University of Technology, Brisbane, Australia), Michael A. Schuetz (Queensland University of Technology, Brisbane, Australia), Nicola Ward (Department of Orthopaedics, Princess Alexandra Hospital, Brisbane, Australia), Michael Wagels (Australian Centre for Complex Integrated Surgical Solutions (ACCISS), Princess Alexandra Hospital, Brisbane, Australia)

Introdution: Lack of sufficient vascularization to support cell viability, growth and function in scaffold guided tissue regeneration (SGTR) is a prevalent challenge facing tissue engineering today. Matching axial vascularization is a regenerative therapeutic approach which incorporates the benefits of flap-based techniques for neo-vascularization to further aid tissue regeneration. Methodology: An ovine critical-size tibial defect model (Medium defect volume=9.5 cm3) was undertaken in eight sheep to evaluate the novel tissue engineering approach involving a 3D-printed medical-grade ε-polycaprolactone b-tricalcium phosphate (mPCL-TCP) scaffold with a cortico-periosteal flap (CPF). Biomechanical, radiological, histological and immunohistochemical analysis confirmed functional bone regeneration comparable to the clinical gold standard control (autologous bone graft) and was superior to a scaffold control group (mPCL-TCP only). A pilot study was performed on two sheep where the defect volume was doubled to 19 cm3 (X-Large defect volume) to represent the most challenging clinical situation. Results: Positive results of both the M and XL defect volume study supported clinical translation. A 27-year-old adult male underwent reconstruction of a 36 cm near-total intercalary defect of the tibia secondary to osteomyelitis using this original scaffold guided bone regeneration concept. Bone regeneration was confirmed postoperatively both radiologically and histologically with complete independent weight bearing achieved within 24 months. Conclusion: This article represents the widely advocated and seldomly accomplished concept of “bench-to-bedside” research. The presented original scaffold-guided treatment concept to reconstruct major bone loss in load-bearing limbs will have significant implications for reconstructive surgery and regenerative medicine more generally.

keywords: bone tissue engineering, critical-sized bone defects, FDA-approved, large animal models, PCL

S27+S56
Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses + Skin wound healing in 2022: where basic science meets clinical needs
Room: S4 B
(29 Jun 2022, 15:30 - 17:00)

Conveners:
Farzaneh Moghtader; Alexandra P. Marque

41883603644

3D IN VITRO M2 MACROPHAGE MODEL TO MIMIC MODULATION OF TISSUE REPAIR

Jiranuwat Sapudom (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Shaza Karaman (Imperial College London, London, United Kingdom), Walaa Mohamed (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Anna Garcia-Sabaté (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Brian Quartey (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Jeremy Teo (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates)

Macrophages are known as the most dominating cells at the wound site, and they coordinate the transition between tissue repair phases during the entire wound-healing process. Especially, anti-inflammatory macrophage (M2) subtypes, namely M2a and M2c, are reported to modulate the tissue repair process tightly and chronologically by modulating fibroblast differentiation state and functions. To establish a well-defined three-dimensional (3D) cell culture model to mimic the tissue repair process, we utilized THP-1 human monocytic cells and a 3D collagen matrix as a biomimetic tissue model. THP-1 cells were differentiated into macrophages and activated using IL-4/IL-13 (MIL-4/IL-13) and IL-10 (MIL-10). Both activated macrophages were characterized by both their cell surface marker expression and cytokine secretion profile. Our results demonstrated that surface markers and cytokines secretion profile of MIL-4/IL-13 and MIL-10 is akin to M2a and M2c macrophages derived from human PBMC, respectively. To mimic the initial and resolution phases during the tissue repair, both activated macrophages were co-cultured with fibroblasts and myofibroblasts. We showed that MIL-4/IL-13 can modulate tissue repair by controlled secretion of TGF-β1 to induce fibroblast differentiation, while MIL-10 macrophages secrete high amounts of IL-10 to resolve inflammation and tissue repair processes. Besides, we demonstrate that IL-10 can reverse myofibroblast into fibroblast phenotypes. By neutralizing IL-10 with antibody in co-culture with MIL-10, no dedifferentiation of myofibroblast could be observed, emphasizing the role of IL-10 in resolution of the tissue repair phase. Overall, our results pinpoint the importance of the co-culture model of fibroblast and macrophages for biomimetic wound healing, instead of fibroblast monoculture. In addition, our established biomimetic model can guide the development of well-defined high-throughput platforms for improving tissue healing and anti-fibrotic drugs testing, as well as other biomedical studies.

keywords: Macrophage, fibroblast differentiation, myofibroblast dedifferentiation, tissue repair, collagen matrix

83767208046

IN VITRO COMPARISON OF SELF-ASSEMBLED AND PLASMA-BASED TISSUE ENGINEERED SKIN SUBSTITUTES: TWO DIFFERENT MANUFACTURING PROCESSES FOR THE TREATMENT OF SEVERE BURN PATIENTS

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Introduction

The use of human bilayer tissue-engineered skin substitutes (hbTESSs) for the treatment of dermatological pathologies is a promising therapy, especially for severe burn patients where there is a lack of donor tissue and wound healing process is disrupted, increasing risk of infection and mortality. In search of personalized medicine, several hbTESSs are under research; their comparison would help understand which technique is more appropriate according to the patient's pathology and condition, however, even at in vitro level, such comparison is complicated because of the high costs or specific requirements. Among hbTESSs, two models have been already applied on more than ten patients as part of respective clinical trials1,2. On the one hand, the Self-Assembly (SA) approach (LOEX-Canada) uses appropriate culture and mechanical conditions to induce fibroblasts to secrete significant amount of extracellular matrix (ECM) as during organogenesis3. On the other hand, human plasma fibrin-based strategy (UPCIT-Spain) generates a dermal layer of fibroblasts embedded into a hydrogel composed, mainly, of human plasma (clotting factor: fibrin) which can be mixed with biomaterials such as hyaluronic acid or collagen4. The aim of this study was to compare these two hbTESSs models.

Methodology

Three human skin samples were collected, and fibroblasts and keratinocytes were extracted for manufacturing both hbTESS models (N=3). Skin substitutes produced by SA approach were composed of three dermal (fibroblasts+ECM) and one epidermal (keratinocytes) layers. Using

human plasma fibrin-based strategy, fibroblasts were embedded into three different dermal matrices (fibrin only, fibrin-hyaluronic acid (HA) or fibrin-collagen (COL)) and epithelialized with a layer of cultured keratinocytes on top. Mechanical properties were analyzed using a tensile testing machine. Immunofluorescence (Ki67, Keratin (K) 19, Collagen-IV, K10, Loricrin), western blot (Collagen-I and -IV) and PrestoBlueTM assay (cell metabolic activity indicator) were performed to compare the results. The same culture media were used for both protocols, but initial number of cells and time of culture followed the original clinical guidelines of each process.

Results
SA approach generates skin substitutes more resistant to tensile forces and with higher adhesion at the dermo-epidermal junction (2 times higher), however plasma-based hbTESSs are thicker, more elastic, and their production is less time-consuming (18 vs. 32 days). Higher number of cells and proliferative cells (Ki67+) is found in SA substitutes although their metabolic activity is lower. After epidermal differentiation, no significant differences were observed between both models, for the number of epidermal stem cells (K19+), and the K10 and Loricrin expression. Overall, production of collagen (I and IV) is higher in SA substitutes, but Collagen-IV is more specifically located at the basement membrane for plasma-based hbTESSs. Finally, properties of plasma-based subtypes are quite similar and only in some specific studies, significant differences are observed (higher amount of Collagen-I in fibrin-COL substitutes -$p<0.01$-).

Conclusions
Our study characterizes two hbTESS models, demonstrating that manufacturing time as well as mechanical and some biological properties are different, however previous clinical studies have already shown their safety. Future in vivo experiments should compare their wound healing potential and long-term persistence after grafting to complete their characterization.

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keywords: Biomaterial, Plasma, Self-assembly, Skin, Tissue Engineering

20941826257

INTRADERMAL ADIPOCYTES DIFFERENTIATION AND LIPID METABOLISM ARE REGULATED THROUGH EPIDERMAL TRANSCRIPTION FACTOR FOXN1

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Introduction. The dermal white adipose tissue (dWAT) is the population of intradermal adipocytes within dermal part of the skin which actively participates in physiological and pathological processes i.e. hair regeneration, thermoregulation, immune response in skin infections and wound healing. Despite the growing interest in this population of adipocytes and identification of their impact on skin physiology, dWAT regulatory pathways have not been fully recognized. It has been shown that activation of epidermal Wnt/β-catenin pathway correlate with dWAT thickness and stimulate adipogenic differentiation by induction of pro-adipogenic ligands: BMP2 (Bone Morphogenetic Protein 2) and IGF2 (Insulin-like Growth Factor 2). Our previous study revealed that epidermally expressed transcription factor Foxn1 regulates homeostasis of epidermis and affects the phenotype and functional characteristics of dermal fibroblasts (DFs). In the present study we investigated the role of transcription factor Foxn1 on intradermal adipocytes differentiation and lipid metabolism in intact and post wounded skin. Methodology. Experiments were performed on young (8-11 weeks old) Foxn1+/+ (Balb /c; with active Foxn1 factor) and Foxn1-/- (with inactive Foxn1 factor) mice. For skin wound healing model, four (4 mm diameter) full-thickness excisional wounds were made on the back of mice. The skin samples were collected from intact skin (day 0) and during the process of healing. At post- injured days 1, 3, 5, 7 and 14, mice were sacrificed and skin samples from the back of the mice were collected using biopsy punches with a diameter of 8 mm. Tissue samples were frozen and stored in liquid nitrogen until RNA and protein isolation or fixed for immunohistochemical and immunofluorescence analyses.

Results. Histological analysis of intact skin showed an increase in the adipocyte number and the percentage of dWAT area in Foxn1+/+ mice compared to Foxn1-/- mice. Immunofluorescence staining pattern of LipidTOX fluorescence dye displayed lipid accumulation exclusively in the lower layer of the dermis, particularly in the skin of Foxn1+/+ mice. The expression profile of genes related to the process of adipogenesis and lipid metabolism demonstrated increased levels of lipolysis markers in Foxn1-deficient mice. Injury increased levels of adipogenic and lipid metabolism genes exclusively in mice with active Foxn1. Interestingly, Foxn1-deficient mice were characterized by lower in comparison to Foxn1+/+ mice expression of adipogenesis regulators (Ppary, Fabp4 and Mest) during the entire healing process. In contrast, the expression levels of lipogenic and lipolytic genes were elevated at later stage of wound healing (day 14) in Foxn1-/- mice in comparison to Foxn1+/+ animals. Western Blot and immunofluorescence analyses of two elements of adipogenic stimulatory pathway revealed higher BMP2 and IGF2 protein content in the skin of Foxn1+/+ mice. Additionally, Foxn1+/+ animals demonstrated peak of Bmp2 expression at 14 day post injury which corresponded with increased Foxn1 mRNA levels during wound healing process.

Conclusions. The results indicate that: (i) Foxn1 modulates dWAT morphology and lipid profile; (ii) stimulated by wounding Foxn1 affects intradermal adipocytes activation during early phase of wound healing; (iii) Foxn1 participates in transcriptional regulation of lipogenesis and lipolysis; (iiii) Foxn1 contributes in stimulation of pro-adipogenic pathways: BMP2 and IGF2.

keywords: dWAT, Foxn1, lipid metabolism, skin wound healing

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DENSE COLLAGEN/PLGA COMPOSITE HYDROGELS GENERATED BY IN SITU NANOPRECIPITATION AS NOVEL MEDICATED WOUND DRESSINGS: IN VITRO AND IN VIVO EVALUATION

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Cutaneous chronic wounds are characterized by the absence of healing after six weeks. The classic treatment is the debridement of the wound bed followed by a compression method. When the treatment is not efficient enough, the application of wound dressings is required. To date, no dressings are appropriated to treat the different kinds of wounds. Nowadays, research orientation is towards medicated wound dressings incorporating therapeutic molecules within biomaterials in order to favor skin repair. In this study, dense collagen/PLGA composite hydrogels have been developed to deliver dexamethasone or spironolactone in a controlled manner to modulate inflammation and favor wound healing. To evaluate composite hydrogels as a novel medicated wound dressing, their physical properties, drug loading and release kinetic were analyzed. Then, the in vivo performance of composites was evaluated in a pig model of impaired wound healing.

Dense fibrillar collagen hydrogels concentrated at 40 mg/mL were incubated in PLGA solutions containing dexamethasone or spironolactone for 24 hours. Different chain lengths from 7 to 60 kDa were tested. Then, the mixtures were incubated in PBS to trigger in situ PLGA nanoprecipitation within the collagen network. The ultrastructure and the mechanical properties of composite hydrogels were analyzed. Last, the drug release kinetic from composites was studied over one month and their cytotoxicity evaluated on fibroblasts and keratinocytes using a live/dead assay. Composite hydrogels loaded with spironolactone were then applied onto full thickness wounds of a pig model. Their effect on wound closure and re-epithelialization was evaluated.

The nanoprecipitation enabled the immobilization of a large amount of PLGA regardless of the chain length (50 % of the total mass). The presence of PLGA negatively impacted the swelling properties but all hydrogels exhibited a high degree of hydration (over 80%). Unlike PLGA 28 and 60 kDa, PLGA 7 kDa did not altered the hydrogel deformability and doubled the hydrogel stiffness. The ultrastructure analysis revealed the presence of polydispersed nano/ microparticles at the surface of collagen fibrils. Compared to pure collagen hydrogels, the drug loading in all composite hydrogels was 5 times higher. The release kinetic of spironolactone and dexamethasone from collagen/ PLGA 7kDa hydrogels was quasi constant over the first two weeks and complete after a month. Unlike pure collagen hydrogels, no burst release was observed. Increasing the chain length negatively impacted the drug delivery as only 20% of the initial dose was released at day 28 for PLGA 60 kDa. Cell viability experiments showed the absence of cytotoxic effect of composite hydrogels on fibroblasts and keratinocytes regardless of the PLGA type used. The in vivo experiment in pig revealed a high performance of collagen/ PLGA composite hydrogels on wound healing. Spironolactone loaded composite hydrogels improved wound closure by 50% and permitted a complete re-epithelialization after 6 days. Taken together, these results show that dense collagen/PLGA composite hydrogels are promising

medicated wound dressings for the treatment of chronic wounds as they deliver constant doses of drugs favoring skin repair, possess good physical properties and promote wound healing in vivo.

keywords: Collagen/PLGA composite hydrogels, nanoprecipitation, drug delivery, wound healing, wound dressing

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HATMSC SECRETED FACTORS IN THE HYDROGEL AS A POTENTIAL TREATMENT FOR CHRONIC WOUNDS—IN VITRO STUDY

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Introduction: Mesenchymal stem cells (MSCs) can improve chronic wound healing, and recently it was suggested that the therapeutic effect of MSCs is mediated mainly through the growth factors and cytokines secreted by these cells. However, MSCs still are not the standard of care in wound healing due to several limitations such as patient-specific difference in MSCs, poor survival of transplanted cells, and technical considerations such as standardization of isolation, characterization, expansion and delivery. To overcome difficulties related to the translation of cell therapy into clinic we propose an innovative, standardized skin treatment option, a conditioned medium (CM) from recently established by our research group Human Adipose Tissue Mesenchymal Stem Cell (HATMSC) line (1). In this study we evaluate the biological activity of HATMSCs-produced factors following incorporation into collagen hydrogel as a potential treatment for chronic wounds (2).

Methodology: Biocompatibility and biological activity of hydrogel-released HATMSC2-origin bioactive factors were investigated in vitro by assessing the proliferation and metabolic activity of human fibroblast, endothelial cells and keratinocytes. Hydrogel degradation was measured using hydroxyproline assay while protein released from the hydrogel was assessed by interleukin-8 (IL-8) and macrophage chemoattractant protein-1 (MCP-1) using ELISAs. Pro-angiogenic activity of the developed treatment was assessed by tube formation assay while the presence of pro-angiogenic miRNAs in the HATMSC2 supernatant was investigated using real-time RT-PCR.

Results: The results showed significant 3-fold increase in metabolic activity of fibroblast ($p < 0.001$) and 2-flold of endothelial cells and keratinocytes ($p < 0.01$) following 3 day culture in the presence of HATMSC2-origin growth factors loaded hydrogels compared to unloaded gels. The supplementation of hydrogel with HATMSCs supernatant improves the tube formation process in angiogenic test in vitro. Moreover, we have confirmed the expression of pro-angiogenic miRNA (miR210, miR126 and miR296) in the HATMSC2 secretome indicating that supernatant can support proangiogenic processes in tissue regeneration. Hydrogel release study showed that there is a substantial difference in the levels of IL-8 and MCP-1 between unloaded hydrogels

and supernatant–loaded hydrogels. For example, on day 3 for MSU-1.1 cells the levels of MCP-1 and IL-8 in hydrogel treated groups were 0.4 pg/mL and 156.2 pg/mL, while in cells treated with supernatant-loaded hydrogel the these level were much higher, 45.5 pg/mL and 1723.7 pg/mL, respectively. This suggest that the hydrogel used in this study is an appropriate carrier of HATMSC-originated trophic factors.

Conclusions: This study demonstrated that the therapeutic effect of the HATMSC2-produced bioactive factors (IL-8, MCP-1, proangiogenic miRNAs) is maintained following incorporation into collagen- hydrogel as confirmed by increased proliferation of skin-origin cells and improved angiogenic properties of endothelial cells. These results suggest the possible beneficial effect of dressing, composed of hydrogel loaded with HATMSCs bioactive factors, on the wound healing process in the context of restoration of proper angiogenesis.

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keywords: adipose tissue-derived mesenchymal stem cells; mesenchymal stem cell secretome; MSC secretome; chronic wound; collagen hydrogel

S28
Emerging and future technologies for peripheral nerve regeneration
Room: S4 C
(29 Jun 2022, 11:00 - 12:30)

Conveners:
Srinivas Madduri

31451701305

UNVEILING THE MULTIPLE ROLES OF STEM CELLS SECRETOME IN NERVE REGENERATION

Antonio Salgado (ICVS, School of Medicine, University of Minho, Braga, Portugal)

The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders, namely due to the beneficial actions of their secretome. Indeed, the latter possesses a broad range of neuroregulatory factors that promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. Considering their protective action in lesioned sites, MSCs, and their secretome, might also improve the integration of local progenitor cells in neuroregeneration processes. In this sense their use could represent an important vehicle for the establishment of future CNS regenerative therapies. In the present talk the role of MSCs, and their secretome, on phenomena such as in vitro and in vivo neuronal/glial survival will be addressed. Additionally, their possible applications, for Spinal Cord Injury and nerve repair be presented. For several years we have been dissecting the role of the secretome of adipose tissue derived stem cells (ASCs), as well as its individual vesicular and proteic individual fractions, in in vitro and in vivo models of axonal growth, inflammation and spinal cord injury, respectively. In vitro experiments revealed that the unfractionated secretome had a significant effect growth, when compared to its protein or vesicular fractions, on axonal growth and neuroinflammatory profile of microglial cells. Following on this data we then evaluated the impact of ASCs secretome on the histological and functional recovery of transection and compression based models of SCI in mice. Results of these experiments revealed that ASCs secretome induced a significant improvement of the locomotor performance of SCI mice, when compared to untreated animals, as assessed by the Basso Mouse Scale test (BMS). This was particular evident in the animals that were injected systemically (IV through the tail veins) with ASCs secretome, when compared to a local delivery. Additionally, the histological analysis has indicated that this motor improvement is closely related with a consistent reduction of the lesion volume, as well as a decreased activation of inflammatory cells (microglia) activation after treatment, as well as an robust increase on the regeneration of new axons. Finally we have also developed approaches for the encpasulation of the secretome in biodegradble systems to facilitate and potentiate their local application.

ACKNOWLEDGEMENTS: Prémios Santa Casa Neurociências - Prize Melo e Castro for Spinal Cord Injury Research (MC-17-2013; MC-04-2017; MC-18-2021); This article has been developed under the scope of the projects NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER). This work has also been funded by FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the projects POCI-01-0145-FEDER-007038 and POCI-01-0145-FEDER-029206.

keywords: Neural regeneration, Stem Cells, Secretome, Hydrogels, Nanoparticles

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THREE-DIMENSIONAL SCAFFOLDS BY MULTI-PHOTON POLYMERIZATION AS A CO-CULTURE SYSTEM FOR TISSUE REGENERATION

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Introduction
Multi-Photon Polymerization (MPL) is a Direct Laser Writing (DLW) technique that combines ultrafast (femtosecond, fs) laser pulses and Computer Aided Designs (CADs) for the fabrication of high precision scaffolds that find application in fields such as tissue engineering [1, 2]. We used such scaffolds for mono- and co- cultures of murine N2a neuronal and SW10 glial cells in order to investigate how topography affects the cell behavior on the 3D environment for various timepoints.

Methods
A novel bridge-shaped 3D designed of dimensions of 400μm x 400μm x 60μm was fabricated using a femtosecond fiber laser operating at 780 nm (pulse duration: 120 fs, repetition rate 80 MHz). The material used for polymerization was a hybrid material consisting of organic and inorganic components (3-Trimethoxysilyl propyl methacrylate, MAPTMS/ Methacrylic acid, MAA and Zirconium propoxide, ZPO). 4,4'- Bis (diethylamino) benzophenon, Bis, and Sudan Black B were both used as photoinitiators (PIs). The fabricated 3D structures were used as scaffolds for the mono- and co-cultures of N2A neuronal and SW10 glial cells for timepoints starting from 7 days. Cultures were monitored both by Scanning Electron Microscopy (SEM) and Confocal Microscopy for both morphological and intra-cellular investigation.

Results
Cell cultures were conducted on both 3D scaffolds and glass coverslips for various timepoints. Cell growth and survival between the different conditions/ culture periods were investigated to determine the optimal culturing conditions. Comparison of the cultures exhibited a strong preference of directionality for cell elongation and axon growth dictated by the topography of the scaffolds compared to the control glass coverslips. Our findings not only show that our scaffolds can sustain both mono and co- cultures of N2a and SW10 cells, but also that by carefully designing a suitable topography, cell behavior can be influenced towards a desired way.

Discussion/Conclusions
Our findings show the effect of topographical properties on cell growth and behavior and the ability to influence the aforementioned behavior in a beneficial way by designing 3D scaffolds with specific geometries based on the application. We highlight the potential of the development of an in vitro model for the study of neurodegenerative diseases which may find further application in tissue regeneration.

Acknowledgments/ Funding
In2Sight: Horizon 2020 GA: 964481

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Comment: Acknowledgments/ Funding
In2Sight: Horizon 2020 GA: 964481

keywords: Multi-photon polymerization (MPP), Tissue regeneration, Co-culture system

94238163729

AN ADVANCED NERVE GUIDANCE CONDUIT FOR REPAIRING LARGE PERIPHERAL NERVE DEFECTS

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Peripheral nerve injuries (PNI) affect millions of patients worldwide and cause motor and sensory dysfunction leading to reduced quality of life and increased healthcare costs. The primary treatment option for repairing large PNIs is to use patient's own nerve graft – an autograft, which is limited by availability and donor site morbidity. In this study, we aim to prepare an off-the-shelf advanced nerve guidance conduit (NGC) with capacity to regenerate critical-sized PNI as effectively, but overcoming the associated limitations of utilising autografts.

Advanced NGCs were composed of two phases – an outer tubular shell composed of collagen type I (CollI) and internal matrix composed of CollI, chondroitin-6-sulphate (CS) that was enriched by adding series of extracellular matrix derived molecules, namely fibronectin, laminin 1 and laminin 2 (CollI-CS-ECM). NGCs with CollI-CS and CollI-CS-ECM were tested for their neurotrophic and immunomodulatory potential in vitro using rat dorsal root ganglia (DRGs) explant culture. Following this, NGCs with CollI-CS (n=16) and CollI-CS-ECM (n=16) and autografts (n=16) were implanted in a large (15 mm) critical-sized rat sciatic nerve defect model.

In vitro analysis showed that in comparison to CollI-CS conduits, CollI-CS-ECM significantly decreased DRGs' secretion of inflammatory markers such as interferon gamma-induced protein 10, monocyte chemoattractant protein 1 and macrophage inflammatory protein 1a and significantly increased DRGs' production of nerve growth factor, vascular endothelial growth factor and interleukin-6.

In vivo analysis showed that sensory and motor function recovery improved significantly over time in all animals. Notably, the response of the two NGCs to electrical stimulation was

similar to the autograft group and no differences were seen in recordings of compound nerve action potential and compound muscle action potential either. Consistent with these results, no significant differences in muscle weight loss were observed between either NGC and autograft group. Importantly, the total area of neurofilament positive staining and the number of myelinated axons within both NGCs was similar to autografts. However, in agreement with our in vitro results, Coll-CS-ECM significantly increased vascularisation inside the conduit when compared to Coll-CS and autograft. This demonstrates the ability of the ECM molecules to direct early regeneration across a large nerve defect.

Collectively, our results demonstrated that enrichment of a NGC with ECM derived molecules such as fibronectin, laminin 1 and laminin 2 resulted in a biomaterial capable of modulating immune response and increasing secretion of pro-repair molecules that ultimately resulted in bridging large PNIs to a level equivalent to an autograft indicating its potential as a new clinical therapy for repairing large nerve defects.

Acknowledgements: This work was co-funded by Science Foundation Ireland and Integra LifeSciences through TP27-1846B1 as part of the Advanced Materials and Bioengineering Research (AMBER) Centre

keywords: extracellular matrix molecules, biomaterial, nerve repair, collagen, animal model

41883656488

ALIGNED AND CONDUCTIVE 3D COLLAGEN/PPY SCAFFOLDS FOR PERIPHERAL NERVE TISSUE ENGINEERING

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Introduction
Peripheral nerve tissue engineering aims to create biomaterials that can replace and possibly even therapeutically surpass the current gold standard nerve autograft. Tissue-engineered constructs can be designed to deliver a combination of benefits to the regenerating nerve, such as supportive cells, alignment, extracellular matrix, soluble factors, and biomechanical integration. An emerging therapeutic opportunity in nerve tissue engineering is the use of electrical stimulation (ES) to modify and enhance therapeutic cell function.

ES has been shown to positively affect four key cell types; neurons, endothelial cells, macrophages, and Schwann cells, involved in peripheral nerve repair1. Briefly, neurons experience faster neurite outgrowth and increased protein adsorption, endothelial cells upregulate angiogenic factors, macrophages may experience a phenotypic shift towards pro-repair phenotype and Schwann cells increase neurotrophic growth factor and exosome secretion. To leverage these phenotypic benefits associated with ES, a conductive tissue engineered scaffold may be used to provide stimulation that improves the regenerative environment, or directly stimulates regenerating axons within the construct. This work attempts to explore how tissue engineering strategies can make use of this therapeutic stimulus to improve nerve regeneration.

Methodology
A novel conductive tissue engineered construct was developed, comprised of conductive organic semiconducting polypyrrole (PPy) nanoparticles distributed within a cellular or acellular collagen matrix, which is then aligned using gel aspiration ejection (GAE) to generate an engineered neural tissue. The GAE technique has been utilized previously for peripheral nerve tissue engineering of cellular collagen gels2 and has therefore been further developed to provide a rapid method to achieve conductive collagen scaffolds in under 1 hour. A fully hydrated hydrogel is aspirated into a cannula, which simultaneously removes the bulk of the interstitial water within the construct and aligns the fibrous collagen with the construct.

Results
The resultant construct is stabilized through this process and due to the conductive PPy nanoparticles distributed throughout the aligned collagen matrix. The material exhibited conductive properties before and after processing with GAE. The conductive engineered tissue was tested in vitro to assess neural cell compatibility and ability of ES to modulate cell phenotype and regeneration.

Conclusions
ES has provided promsing results to short nerve gap injuries. This approach provides a promising new method for investigating whether ES can be used to enhance nerve tissue engineering, and importantly address clinical need within 'critical length' nerve injury gaps.

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keywords: Conductive, Bioelectricity, Tissue Engineering, Scaffold

41883636477

EXTRACELLULAR VESICLES IN PERIPHERAL NERVE REGENERATION: EXTRACELLULAR VESICLES DERIVED FROM ADIPOSE STEM CELLS INCREASE SCHWANN CELL PROLIFERATION FOLLOWING INTERNALIZATION

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Introduction. Extracellular vesicles (EVs) are involved in a plethora of physiological and pathophysiological contexts, and their potential regenerative applications have attracted special interest. Ease of autologous isolation, low immunogenicity and lack of reproductive potential are only some of the enticing characteristics that turn the spotlight increasingly towards EV-based therapy. However, too many unknowns regarding the biology of EVs remain. Within the scope of our research, we focus on peripheral nerve regeneration, where we challenge the gold standard of autologous reconstruction with alternative therapeutic approaches, including EVs. Schwann cells (SCs) have been ascribed an essential role in nerve repair. Their response to nerve damage includes debris clearance, attraction of macrophages, and providing structural and trophic support for the regrowing axon. In this study, adipose tissue derived stem cells (ASCs) serve as a source for EVs. Here, we follow the journey of ASC-EVs to the recipient SCs and decipher how they are able to transmit their multifaceted signals and actuate downstream processes, including proliferation.

Methods. EVs were isolated by differential ultracentrifugation and characterized according to the MISEV guidelines. Imaging flow cytometry (IFC) allowed immunophenotyping with a single-vesicle resolution, while nanoparticle tracking analysis (NTA) was used to determine concentration and size distribution. The entire SC – ASC-EV interaction was observed with live-cell imaging (LCI), from initial contact to subsequent internalization and perinuclear translocalization, which was confirmed with 3D image reconstructions of high-resolution confocal micrographs. Scanning electron microscopy enabled us to elucidate the initial contact in detail, while transmission electron microscopy granted us a closer look at the vesicle transit through the cellular membrane. We further broke down the membrane transit on a molecular level by pairing well-established pharmacological inhibitors of major endocytotic mechanisms with state-of-the-art IFC. The cellular response to ASC-EV treatment was quantified via EdU incorporation during DNA synthesis.

Results. Upon initial contact with SCs, ASC-EVs were moved along the membrane until they were internalized and subsequently transported towards the cell's nucleus, where they were accumulated. The inhibition of specific endocytosis pathways revealed that in SCs, the internalization of ASC-EVs is mainly mediated by clathrin, though alternative modes of membrane transit are likely involved, as no complete block of ASC-EV-internalization could be achieved. Upon internalization of ASC-EVs, we observed an increase in SC proliferation in a time- and dose-dependent manner, up to 2.5-fold compared to untreated SCs within 72h.

Conclusions. We established that ASC-EVs can enhance proliferation in SCs, crucial for peripheral nerve regeneration. This response is activated upon internalization of ASC-EVs. We identified the

major mode of internalization, however, alternative modes of internalization likely involved. The potential therapeutic application of EVs necessitates understanding the underlying processes, especially the interaction with target cells. Our investigations provide a deeper understanding of the cellular signal transduction during peripheral nerve regeneration upon stimulation with ASC-EVs and adds to the knowledge needed to harness the full potential of EVs for therapeutic purposes.

keywords: extracellular vesicles, schwann cells, endocytosis, adipose stem cells, nerve regeneration

S30
European regional platforms for TERM - Update
Room: S4 C
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Gerjo van Osch; Heinz Redl

62825438124

REGENERATIVE MEDICINE AND TECHNOLOGY – A NEW BACHELOR PROGRAM

Guy Bendermacher (Maastricht University, Maastricht, Netherlands), Lorenzo Moroni (Maastricht University, Maastricht, Netherlands), Jurica Bauer (Maastricht University, Maastricht, Netherlands)

Introduction:
Regenerative Medicine is a relatively new field found at the intersection of science, engineering and medicine. Researchers in this field are traditionally biologists, chemists, materials scientists, data scientists, engineers or physicians who have acquired skills and knowledge beyond their basic training and stepped into the rapidly evolving field of TERM. Many of them, however, still find themselves approaching their research questions from the perspective of their own traditional discipline finding it difficult to adopt the much needed multidisciplinary approaches. Having recognized the need for a new researcher profile that would receive basic training in multiple disciplines, at Maastricht University we have taken the initiative of designing and developing an undergraduate program focusing on Regenerative Medicine and Technology. Within this program we aim to educate a new generation of researchers that will be able to swiftly adapt to any area of TERM, and contribute to the design and development of medical therapies, products and devices for research and clinical use.

Methodology:
A multidisciplinary Curriculum Committee and a broader Consultative Committee were assembled with the goal of developing an outline of a possible curriculum for the envisaged program using the principles of backward chaining. A total of nine Developing Groups have been installed to develop and constructively align the first two years of education keeping the CCCS (Constructive, Contextual, Collaborative, Self-Directed) learning principles in mind.

Results:
Applying the principles of problem- and research-based learning our program intends to offer a solid science and engineering foundation which would be strongly integrated with the relevant aspects of medicine. To this end, the desired competencies have been defined and in turn translated to year intended learning outcomes and a three-year curriculum. The first year offers a strong foundation in science, engineering and regenerative medicine. The second year is intended to focus on application of the acquired foundation in TERM. An example of this is a real-world student research project defined by clinicians within the MUMC+ university hospital. In addition, special attention will be paid to the development of translational science skills necessary to bring new therapies and products onto the market. We also envisage a close collaboration with the industry to bring in real-life success and fail stories. The third year would offer a minor and a semester-long research graduation project in TERM. The program aims to integrate the perspectives and input of the TERMIS-EU community and holds the ambition to catalyze the creation of an international network in education.

Conclusions:
The design and development of a new undergraduate program with the focus on TERM has been initiated at Maastricht University. The first ideas on the competencies and final qualifications have been translated into a curriculum outline draft. The education will be developed in further detail keeping the principles of constructive alignment and the CCCS learning principles in mind. The subsequent steps to be taken are the preparation of a macro-efficiency file and, in case of a

positive evaluation, also an initial accreditation procedure. The program's envisioned start date would then be September 2023.

keywords: Regenerative Medicine, Technology, Education, Bachelor Program

S31
Extracellular vesicles – next generation tool for diagnostics and regenerative medicine
Room: S3 A
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Ewa Zuba-Surma; Barbara Łukomska

94355106237

MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES AND THEIR FUNCTIONAL HETEROGENEITY

Bernd Giebel (Institute for Transfusion Medicine; University Hospital Essen, Essen, Germany)

Human mesenchymal stem/stromal cells (MSCs) are a therapeutically relevant, heterogenous cell entity with immunomodulatory and pro-regenerative potentials. Apparently, MSCs mediate a huge proportion of their therapeutic effects via extracellular vesicles (EVs). Connected to several advantages in using cell-free products for the therapeutic setting, MSC-EVs emerged as promising novel therapeutic agent for various diseases, including graft-versus-host disease (GvHD), ischemic stroke, COVID-19 and sepsis.
It is our current mission to optimize the MSC-EV production strategy in a scaled, GMP compliant manner, and to set up an appropriate quality control platform to translate MSC-EVs into the clinics. One of the challenging aspects in this context is inherited from the MSC field, i.e. contradictory reports on the efficacy of MSC therapies. Apparently, not all MSC products mediate therapeutic effects when applied into patients. Similarly, we observe functional differences among independent MSC-EV preparations; even when same MSC stocks were used as starting material. Thus, to avoid draw backs as they occurred in the MSC field by failing to show efficacy in a phase III clinical trial for GvHD treatment, it is one of our most important missions to address the heterogeneity aspect by establishing appropriate read outs and set up strategies for allowing scaled and reproducible manufacturing of potent MSC-EV products.

keywords: Extracellular vesicles, Exosomes, Mesenchymal stromal cells, stem cells

41883634604

EXTRACELLULAR BIOADDITIVES-ADJUVANTED INJECTABLE HYDROGEL SUPPORTS NEOANGIOGENESIS AND DAMPENS ADVERSE CARDIAC REMODELLING

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In the last years, the role played by extracellular vesicles (EVs) in inter-cellular and inter-organ communication through the delivery of signal molecules has been revealed and, nowadays, it is considered to be of utmost importance. EVs are secreted by almost all cell types and have an important role in several research fields ranging from oncology to immunology and diagnostics to regenerative medicine. However, the therapeutical employ has not yet been established, although their enormous biological potential is well known. The major limitation to EVs clinical use is the inability to localize the in vivo benefits into strategically defined sites of interest to avoid side effects. Numerous recent discoveries have shown how injectable hydrogels can be used for biomedical applications in regenerative medicine. Specifically, hydrogel-based drug delivery systems are found to be more efficacious than the conventional systemic administration.

Based on these premises, we proposed a new strategy to harness stressed endothelial cell-derived EVs and their angiogenic cargo in injured tissues. EVs, derived from human endothelial cells were collected and used as bioadditives for Gelatin Methacrylate (GelMA) formulation and functionalization. Our results showed that 3D bioprinted structures loaded with EVs support the formation of a functional neovascular network in-situ, constituted of perfused microvessels recapitulating the print pattern. In addition, we assessed the EVs-GelMA effects on cardiac remodeling after acute myocardial infarction (AMI). For this purpose, the EVs-GelMA was injected and polymerized into the ischemic ventricular cardiac wall after the surgical induction of AMI. Results showed improved cardiac performance and a reduction of the ischemic area with partial revascularization of the cardiac wall in the treated animals. Taken together, these findings support the development of new advanced regenerative applications for the revascularization of ischemic organs and peripheral tissues.

keywords: extracellular vesicles, bioadditives, hydrogel, regenerative medicine

31412704386

EXTRACELLULAR VESICLES FROM HUMAN IPS CELLS ENHANCE RECONSTITUTION CAPACITY OF CORD BLOOD-DERIVED HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Introduction: Human cord blood (CB) represents a rich source of several stem cell (SCs) types including hematopoietic stem and progenitor cells (HSPCs). Thus, clinical application of CB cells has become an alternative for the bone marrow transplantation. However, successful application of CB-HSPCs in adult patients requires the development of effective strategies improving their ex vivo expansion, homing and regenerative potential. One of the promising approaches for enhancement of SCs functionality includes their treatment with extracellular vesicles (EVs), which were shown to harbor and transfer bioactive content. Thus, in our study, for the first time we have evaluated an impact of human induced pluripotent SCs (hiPSCs)-derived EVs (hiPSC-EVs) on selected functions of CB-HSPCs, important for their hematopoietic potential in vitro and in vivo.

Methodology: hiPSC-EVs were harvested from media collected from feeder-, serum- and xeno-free cultures of hiPSCs by sequential ultracentrifugation. Next, CD45dimLin-CD34+ cell fraction enriched in HSPCs was isolated from CB by magnetic- (MACS) and fluorescence-activated cell sorting (FACS) and further expanded in dedicated serum- free media. Subsequently, we evaluated the influence of hiPSC-EVs on several biological and functional properties of CB-HSPCs in vitro and in vivo.

Results: Our results revealed that hiPSC-EVs may transfer their bioactive content and improve functional properties of CB-HSPCs including metabolic activity, hematopoietic and clonogenic potential, as well as survival, chemotactic response to stromal cell-derived factor 1 (SDF-1) and adhesion to the model components of hematopoietic niche in vitro. Importantly, hiPSC-EVs enhanced homing and engraftment of CB-HSPCs in vivo. Additionally, we have demonstrated that the treatment with hiPSC-EVs may activate signalling pathways in CB-HSPCs on both gene expression and the protein level.

Conclusion: In conclusion, our findings suggest that the “priming” with hiPSC-EVs may improve several functions of CB-HSPCs important for their homing and hematopoietic activity following the transplantation. These results support the new concept envisioning hiPSC-EVs as next-generation tools that may enhance future applications of CB in hematology.

Comment: Acknowledgements: This study was funded by NCBR grant STRATEGMED III (STRATEGMED3/303570/7/NCBR/2017) and NCN grant MAESTRO 11 (2019/34/A/NZ3/00134) to EZS and Young Scientist FBB&B GRANT (BMN 19/2016) to EK.

keywords: bone marrow reconstitution, cord blood, extracellular vesicles, hematopoietic stem and progenitor cells, hiPSCs

41883644586

INTRA-TRACHEAL INJECTION OF HUMAN EXTRACELLULAR VESICLES BLOCKS FIBROSIS AND REGENERATES EPITHELIAL LUNG CELLS IN A RAT MODEL OF BRONCHOPULMONARY DYSPLASIA

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INTRODUCTION: Bronchopulmonary Dysplasia (BPD) is a life-threatening disorder affecting premature newborns, for which no definite cure is available1,2. Lung fibrosis is one of the main problems that affect young patients. The aim of this work was to investigate the mechanism of action of human extracellular vesicles (EVs) both in vitro and in an animal model of hyperoxia-induced BPD. Specifically, we evaluated the effects of EVs on the development of fibrosis and on functionality of lung epithelial cells.

METHODOLOGY: GMP-grade EVs were produced by human Wharton-Jelly derived MSCs (Exo Biologics, Belgium), isolated by tangential flow filtration and characterized according to MISEV2018. Rat pups were divided in 3 groups: normoxia + PBS vehicle (control group), hyperoxia with PBS (untreated), hyperoxia with MSC EVs in PBS (treated). Both PBS and EVs were injected intratracheally (IT) on day 3, 7 and 10 and pups were sacrified on day 14. The expression of the genes TGFβ1 and aSMA was analysed in lungs. To evaluate epithelial secretory function, the expression of glycosaminoglycans (Alcian blue staining) and of surfactant protein C (SFTPC) was analyzed by histology and immunofluorescence. Collagen deposition was assessed by Sirius Red staining. Macrophages from rat bone marrow were treated with TGFβ, cultured and analyzed for aSMA and CD90 expression by flow cytometry.

RESULTS: Pups under hyperoxia exhibited an increase in collagen deposition in the lungs. This parameter was reduced by treatment with MSC EVs. The area of lung tissue expressing glycosaminoglycans was significantly increased in MSC EV-treated rat pups in respect to untreated animals. In addition, cells expressing SFTPC were significantly increased in MSC EVs treated pups with respect to the untreated group. In vitro, MSC EVs suppressed the induction of aSMA expression in macrophages.

CONCLUSIONS: Intratracheal administration of clinical-grade MSC-EVs counteracts the development of fibrosis and improve pulmonary epithelial function in a neonatal model of hyperoxia-induced lung injury. These results can contribute to understand the mechanism of action of these nanoparticles in preventing the development of BPD.

1- Porzionato, A. et al., Am J Physiol Lung Cell Mol Physiol 316: L6–L19, (2019)
2- Hansmann, G. et al., Pediatric Research 89:446 – 455, (2021)

keywords: Extracellular Vescicles, fibrosis, Bronchopulmonary Dysplasia

20941834084

DEVELOPMENT OF BIOINSPIRED PROTEOLIPOSOMES AND CELL-DERIVED NANOVESICLES AS OSTEOGENIC SYNTHETIC EXTRACELLULAR VESICLES FOR BONE REGENERATION

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In regenerative medicine, extracellular vesicles (EVs) have been increasingly studied as alternative acellular therapies overcoming the limitations of cell-based strategies. Derived from mineralising osteoblasts, EVs demonstrated their osteogenic potency suggesting their potential as a novel bone regenerative therapy. However, the clinical translation of EVs remains limited by issues associated with the scalability, reproducibility and purity of these naturally-derived nanoparticles. In this study, the characterisation of mineralising-osteoblast-derived EVs (MO-EVs) was performed to inspire the development of osteogenic synthetic EVs.
EVs were collected from cultures of mineralising osteoblasts over a 2-week period and the EV-isolation was performed by ultracentrifugation. Subsequently, the size, ζ-potential, morphology and particle concentration of these nanovesicles was characterised and the presence of tetraspanin markers (CD9, CD63 and CD81) was confirmed using nano-flow cytometry. Furthermore, the pro-osteogenic capacity of MO-EVs was assessed in vitro via quantifying alkaline phosphatase (ALP) activity and calcium deposition. From the composition analysis of MO-EVs, bio-inspired proteoliposomes harboring ALP and/or annexin VI were formulated via the thin-film hydration method followed by extrusion. Both the activity of the proteins post-insertion and their resulting incorporation efficiency in proteoliposomes were then determined. Additionally, cell-derived nanovesicles (CDNs) were produced by the serial extrusion of mineralising osteoblasts and the resulting synthetic EVs were similarly characterised.
The isolation of MO-EVs was validated as positivity for all tetraspanin markers was reported for these sub-100 nm vesicles. Notably, their osteogenic potency was confirmed in vitro on osteoblasts as MO-EVs increased significantly ALP activity, calcium deposition and collagen production after a 2-week treatment. MO-EVs were found enriched in several annexin proteins which guided the formulation of bio-inspired proteoliposomes. The insertion of both ALP and annexin VI was successful with >30% incorporation efficiency obtained for all formulations. Importantly, EV-inspired liposomes harboring annexin VI or ALP were found to be functional with the validation of the mediation of Ca^{2+}-influx by annexin VI inside proteoliposomes and the confirmation of ALP enzymatic activity. Moreover, CDNs were also successfully produced as a nanoparticle population with an EV-size was obtained after serial extrusion. Both EV-inspired proteoliposomes and CDNs' osteogenic potencies were then compared to MO-EVs after 14 days in osteogenic conditions.
Taken together, these results shows the potential of the development of synthetic EVs as biomimetic nanocarriers to accelerate the clinical translation of EV-based therapies for bone regeneration.

keywords: Extracellular vesicles, synthetic EVs, liposomes, cell-derived nanovesicles, bone regeneration

52354522124

SECRETOME OF ADIPOSE TISSUE DERIVED STEM CELLS AND ELECTRICAL EPIDURAL STIMULATION PROMOTES FUNCTIONAL GAINS IN SPINAL CORD INJURY CONTEXT

Jorge Ribeiro (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal), Deolinda Silva (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal), Tiffany Pinho (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal), Supti Bhattacharyya (Faculty of Biological Sciences, University of Leeds, Leeds, UK, Leeds, United Kingdom), Serra BSc (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal), Nuno Silva (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal), Ronaldo Ichiyama (Faculty of Biological Sciences, University of Leeds, Leeds, UK, Leeds, United Kingdom), António Salgado (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, Braga, Portugal)

Spinal cord Injury (SCI) is a life changing event with a high number of new cases reported every year. The most common cause of SCI comes from traumatic events, such as traffic accidents, falls, violence and sports activities, while the non-traumatic events (tumours, neurodegenerative and infectious diseases) are less prevalent. The injury incurred in the spinal cord tissue triggers several pathophysiological events which cause damage and supress axonal growth in the spinal cord tissue. These events consist of activation of apoptotic pathways, the release of inflammatory cytokines and the formation of a glial scar that primarily contains further damage, but also releases biomolecules that inhibit axons outgrowth. It affects nerve fibres passing through the lesion site causing motor dysfunction and altered sensation, causing incapacitating conditions to SCI patients. In this work we suggest a new multidisciplinary approach, combining the value of Adipose Tissue Derived Stem Cells (ASCs) secretome and Electrical epidural stimulation (EES). ASCs secretome was reported to promote regaining of function after SCI in a mouse model, likely promoted by the factors present in it which include anti-apoptotic and angiogenic factors, neuroprotectants and immunomodulators which may prime the unfavourable environment created upon SCI to a more neuroprotective/regenerative one. These benefits combined with the EES, which promote spinal cord plasticity, central pattern generator activation and stepping initiation is expected to improve functional gains after SCI. In an organotypic model of ex vivo spinal cord, we have observed that the secretome from ASCs is a potent modulator of axonal growth and inflammatory cells migration. We observed an increase in neurite outgrowth as well as migration of iIba-1+ cells to the outside of the explant ($P<0.05$). This confirms the paracrine effect of the secretome in the spinal cord environment with a potential translatable effect in vivo. On this wise, we decided to combine the ASCs secretome with EES in a rat model of SCI (severe contusion) in vivo. We observed a synergetic effect of both treatments on the locomotor score (BBB scale), body weight support, maximum speed, number of steps and dragging time during the stepping cycle, with the combinatory approach demonstrating superior performance than control rats in all parameters analysed. We also detected an interesting result in the Randall Sellito test (pain response), which may suggest an increase in intraspinal plasticity after the treatment. Altogether, this provides evidence of the therapeutic potential of ASCs secretome after SCI, supported by indications on the positive effects exerted on neuroinflammation, axonal outgrowth and regeneration in vitro. This potential is also highlighted when combined with EES in vivo, with functional gains observed in the locomotor performance when compared to control.

keywords: Adipose mesenchymal stem cells; Secretome; Eletrical Epidural Stimulation; Spinal cord Injury

S34
Advanced therapy approaches in tissue engineering
Room: S4 A
(1 Jul 2022, 11:00 - 12:30)

Conveners:
Lara-Saez; Wenxin Wang

73387306587

NON-VIRAL GENE DELIVERY PLATFORM FOR TOPICALLY TREATING RARE GENODERMATOSES

Wenxin Wang (University College Dublin, Dublin, Ireland), Irene Lara-Saez (University College Dublin, Dublin, Ireland), Xianqing Wang (University College Dublin, Dublin, Ireland), Yinghao Li (University College Dublin, Dublin, Ireland)

Gene therapy is the most promising treatment for recessive dystrophic epidermolysis bullosa (RDEB), however genetic cargo delivery efficiency is still a technical limitation. Viruses are the traditional vector of preference for gene therapy, as virus trophism increase tissue specificity. However, drawbacks related with safety and high manufacturing costs have facilitated the expansion of non-viral vectors, such as liposomes and cationic polymers. Our group is focused on the development of highly branched cationic polymers for gene therapy to treat RDEB. Our polymers have demonstrated encapsulation and delivery of a full COL7A1 cDNA with no toxicity, performing better transfection efficiencies than commercial counterparts in RDEB keratinocytes. In this work, we show the research progress expanding the polymer technology for mRNA, pDNA and ribonucleoprotein complex delivery for developing CRISPR/Cas9 based gene editing therapies for RDEB. Gene edition in vivo has been achieved by a single topical application, obtaining efficiencies comparable with viral vectors (Ad5). Endosomal escape, by peptide polymer decoration is being investigated to improve efficiency in vivo by avoiding endosomal retention. Storage of the nanoparticles, formed by polymers and the genetic cargo, at -20C ensures no reduction in efficiency for more than 6 months. However, in order to avoid cold chain challenges, nanoparticles have been lyophilised, increasing dose concentration and facilitating formulation with skin absorption enhancers for topical application.
Proven ability to transfect stem cells combined with high efficiencies transfecting with multiple plasmids at the same time, should contribute to the success of prime editing strategies to pursue permanent correction of potential >89% of all described EB disease-associated mutations. The developed polymer platform shows high potential to be adapted to a wide range of genetic approaches for RDEB, including the most novel ones, that can be expanded to other EB subtypes, other genodermatoses and other rare genetic disorders such as cystic fibrosis.

keywords: Epidermolysis bullosa, cationic polymers, gene therapy

52354518008

DEVELOPMENT OF COLLAGEN-NANOHYDROXYAPATITE SCAFFOLD PLATFORM FOR DUAL-DELIVERY OF A MICRORNA-26A MIMIC AND MICORORNA-133A INHIBITOR FOR TREATMENT OF LARGE VOLUME BONE DEFECTS

Joanna Sadowska (Royal College of Surgeons in Ireland, Dublin, Ireland), Austyn Matheson (Royal College of Surgeons in Ireland, Dublin, Ireland), Monika Ziminska (Queen's University Belfast, Belfast, United Kingdom), John Redmond (Dublin City University, Dublin, Poland), Nicholas Dunne (Dublin City University, Dublin, Ireland), Helen McCarthy (Queen's University Belfast, Belfast, United Kingdom), Sam Wojda (University of Massachusetts Amherst, Massachusetts, United States), Cole Ferreira (University of Massachusetts Amherst, Massachusetts, United States), Seth Donahue (University of Massachusetts Amherst, Massachusetts, United States), Fergal O'Brien (Royal College of Surgeons in Ireland, Dublin, Ireland)

INTRODUCTION: There remains a substantial unmet clinical need for tissue engineered strategies to heal large volume bone defects. The delivery of microRNAs from biomaterial-based scaffolds presents a promising approach: whereby the scaffold provides a structural support to bone tissue while the microRNAs (miRs) induce the endogenous cells to produce relevant therapeutic proteins and genes at physiological levels while shutting off aberrant effects 1,2. However, the effective delivery of miRs is frequently jeopardized by their poor stability, requiring a suitable vector which would protect them from degradation guaranteeing their effective intracellular delivery and transient secretion of osteogenic proteins by host cells. In this study, collagen-nanohydroxyapatite (coll-nHA) scaffolds1,2,3, previously optimized for bone repair within our lab, were coupled with self-assembling, amphiphilic, cell-penetrating RALA peptide4 as a delivery non-viral vector yielding a scaffold-based system for simultaneous delivery of miR-26a mimic1,2 and miR-133a inhibitor5 for bone repair.

METHODS: miRs were complexed with cationic RALA peptide5, incorporated (1µg or 3 µg) into coll-nHA scaffolds1 which were assessed in terms of calcium release, loading efficacy, distribution and release of nanoparticles (NPs). 3×105 human mesenchymal stem cells (hMSC) were seeded onto the miR-activated scaffolds and the expression of miRs, metabolic activity, DNA content, ALP activity and calcium deposition were quantified. The scaffolds were implanted into calvarial defect in male rats, the total bone volume and tissue mineral density were assessed at week 4, 8 and 12 of the study.

RESULTS: The NPs were successfully incorporated into scaffolds and worked effectively delivering miRs to the hMSCs in controlled manner. The miR-activated scaffolds cultured in cell-free media showed sustained release of miRs, uptake of calcium, and an increase in compressive modulus. The scaffolds delivered the miR-26a mimic or miR-133a inhibitor, either alone or combined, to the hMSCs resulting in a silencing effect and an enhanced ALP activity. The miR-activated scaffolds enhanced the healing in rat calvaria generating greater amount of bone compared to the scaffold alone.

CONCLUSION: This study describes the development of scaffold system using self-assembling, amphiphilic, cell-penetrating peptide for sustained delivery of therapeutic microRNAs for treatment of bone defects. The miR-activated scaffolds transfected the hMSCs with miRs enhancing the osteogenesis of the cells3,5. The miR-scaffold system has potential to be used as a next generation therapeutic for repair of large bone defects offering precise and transient gene editing with minimal immunogenicity. The novel miR co-delivery scaffold-based system is

versatile and has the potential for a myriad of applications beyond bone repair by tailoring the individual miRs delivered – as well as the scaffold composition.

ACKNOWLEDGEMENTS: National Science Foundation- Science Foundation Ireland (NSF-SFI) US-Ireland R&D Partnership Programme (NSF_ 17_US_3437). JMS benefits from a Marie Skłodowska-Curie Individual Fellowship from the European Commission through the H2020 project GAMBBa (Project ID: 892389).

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keywords: bone regeneration, gene delivery, microRNA, scaffolds

41883619327

CYSTIC FIBROSIS: REGENERATING LUNG EPITHELIAL CELLS FUNCTION WITH NON-VIRAL GENE THERAPY

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Introduction:
Cystic fibrosis (CF) is a lethal autosomal recessive inherited disease caused by mutations in the CFTR gene encoding the CF transmembrane conductance regulator (CFTR) protein, and has no cure to date. CFTR gene mutations lead to abnormal chloride ion transport in epithelial tissues, which changes the hydration and pH of fluids and mucus, affecting respiratory (main cause of morbidity and mortality) and digestive systems among others [1].
Gene therapy is the most suitable approach for treating this disease. Gene replacement strategies for CF have been previously developed but failed due to low gene delivery efficiency combined with brief expression in epithelial cells. Although gene therapy based on viral vectors has been proven to be efficient, safety risks and immune response are important limitations [2]. The rise of non-viral vectors has taken place to overcome these drawbacks, however, clinical trials with liposomes have been performed and finally abandoned.
In this work, we propose a different approach that consists in the development of a non-viral gene therapy based on highly branched poly(β-amino ester)s (HPAE), which family has been previously shown to perform high transfection efficiency in other genetic conditions [2], and offers advantages compared to liposomes such as easier functionalization and higher stability and retention of the cargos [3].

Methodology:
A library of cationic HPAE polymers was developed, characterized, and optimized by selection of the best molecular weights, enhancement of the branched structure and the biocompatibility, and terminal group performance evaluation. Then, a screening of the candidate polymers was carried out in vitro in CF disease model cell lines in terms of cell viability and plasmid DNA transfection efficiency. Moreover, optimized CFTR gene-containing DNA plasmids were constructed to be combined with the HPAEs developed.

Results:
A family of HPAEs with different monomeric combinations and terminal groups was created and their physicochemical features were characterized. Among all these polymers developed for plasmid DNA delivery, some of them showed higher transfection efficiency than others commercially available in CF lung epithelial cell lines, with similar or higher levels of cell viability. In addition, different CFTR gene-containing DNA plasmids with different combinations of promoters and enhancers were successfully obtained as an alternative system to the gene replacement strategies currently available.

Conclusions:
The basis for the development of a promising non-viral therapy for CF has been laid. This has been possible thanks to the synthesis of efficient gene delivery tools as are HPAE polymers combined to optimized genetic systems to restore the CFTR protein levels, regenerating thus

the lung epithelial cells function. This approach offers a new perspective from the clinical trials performed to date with other non-viral vectors and is expected to be further tested in vivo as an inhalation therapy. In addition, these systems have got a high potential for future commercialization and as bench-to-bedside research.

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keywords: Gene therapy, cystic fibrosis, polymers, DNA

20941856088

NANOPARTICLE-MEDIATED SELECTIVE SFRP-1 SILENCING ENHANCES BONE DENSITY IN VIVO IN OSTEOPOROTIC MICE BY THE STIMULATION OF THE CANONICAL WNT/B-CATENIN PATHWAY

Patricia Diaz-Rodriguez (Universidade de Santiago de Compostela, Santiago de Compostela, Spain), Patricia Garcia-Garcia (Universidad de La Laguna, La Laguna, Spain), Ricardo Reyes (Universidad de La Laguna, La Laguna, Spain), Carmen Évora (Universidad de La Laguna, La Laguna, Spain), Araceli Delgado (Universidad de La Laguna, La Laguna, Spain)

Introduction: Osteoporosis (OP) is characterized by a loss in bone mass and mineral density1. The stimulation of the canonical Wnt/β-catenin pathway has been reported to promote bone formation by increasing the osteogenic potential of mesenchymal stem cells (MSC)2. This pathway is controlled by several regulators as secreted frizzled-related protein-1 (Sfrp-1) that acts as an antagonist3. Thus, Sfrp-1 silencing therapies could be suitable for enhancing bone formation. However, the stimulation of this pathway at a systemic level has been correlated with adverse cardiovascular events. The use of nanoparticles (NPs) for oligonucleotide targeted delivery could be effective avoiding these undesirable effects. Aptamers (Apt) can recognize and bind specific structures based on their three-dimensional conformation. This work hypothesizes the systemic administration of lipid-polymer NPs (LPNPs) functionalized with a MSC specific Apt and carrying an SFRP1 silencing GapmeR, could favor bone formation in OP.

Methodology: Different pegylated-LPNPs formulations were prepared and the effect of pH, GapmeR encapsulation and aptamer functionalization on their physicochemical properties was evaluated by DLS and TEM. The oligonucleotide encapsulation efficiency and its in vitro release at variable temperatures were evaluated using fluorescently-labeled GapmeR. Adequate SFRP1 GapmeR-loaded Apt-LPNPs (Apt-LPNPs-SFRP1) were then evaluated in terms of cytocompatibility and gene silencing efficiency. Finally, the developed systems were administered in vivo in osteoporotic mice and their biodistribution and bone induction capacity was evaluated using radiolabeled LPNPs or by the femurs' histological, histomorphometric and immunohistochemistry assays after three months, respectively.

Results: The developed LPNPs show an adequate average diameter of approximately 160 nm independently of the GapmeR encapsulation. Moreover, these formulations presented low polydispersity indexes (< 0.3). The incorporation of the aptamer in the nanoparticles surface, as expected, led to a decrease in the ζ-potential. Both LPNPs-SFRP1 and Apt-LPNPs-SFRP1 exhibited a spherical core-shell structure, characteristic of LPNPs. Their GapmeR encapsulation efficiency was 64 ± 4.32 % for both LPNPs showing a biphasic release pattern. The treatment of MSCs with LPNPs at variable concentrations did not show any toxicity. On the other hand, the treatment of MSCs with LPNPs-SFRP1 decreased the sfrp1 expression but less than the positive control (Damafect). Nevertheless, for cells treated with Apt-LPNPs-SFRP1, sfrp1 expression levels were similar to positive control. Moreover, the aptamer functionalization modified the LPNPs biodistribution profile showing a four-fold increase in the bone accumulation and a ten-fold decrease in the hepatic accumulation compared to naked LPNPs. The femurs histological evaluation revealed evident changes in bone structure and microarchitecture observing a more compact trabecular bone and a cortical bone thickness increase, in the Apt-LPNPs-SFRP1 treated mice compared to control (saline solution). Moreover, the immunohistochemical analysis of Col-I and OCN, revealed increased immunoreactivity for both markers in the Apt-LPNPs-SFRP1 treated mice.

Conclusions: Aptamer functionalized LPNPs loaded with SFRP1 silencing GapmeR showed adequate properties and biodistribution profiles leading to an enhancement on the bone density of osteoporotic mice.

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keywords: bone regeneration, osteoporosis, gene therapy, nanoparticles

62825446719

MICRORNAS AND THEIR ROLE IN MULTIPLE TRAUMA: PROFILING LOCAL AND SYSTEMIC EXPRESSION LEVELS

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Trauma is the leading global cause of mortality and disability. Patient management after trauma can be challenging, particularly in a multiple trauma setting, a condition characterized by several severe injuries. Multiple trauma can elicit major immunological responses, such as systemic inflammatory response syndrome, which lead to a deterioration in the patient's condition. The complement system, being a central component of the immunological response after trauma, plays an important role therein. The activation of the complement system in multiple trauma, and subsequent regulation of inflammatory cascades, are therefore of clinical interest. MicroRNAs, important post-transcriptional gene regulators, may play a regulatory role in the activation and progression of immunological and regenerative responses in multiple trauma. The aim of this study was therefore to examine microRNA expression in the systemic circulation, and the sites of injury, in a porcine multiple trauma model. In this model, two different trauma treatment methods were compared, as well as one separate treatment group which was administered a combination of a C5-convertase inhibitor to inhibit complement system activation, and an anti-LPS receptor tore inhibit PAMP activation pathways.

The porcine multiple trauma model consisted of blunt chest trauma, liver laceration, bilateral femur fracture, and controlled haemorrhagic shock. Animals were operatively and medically stabilized, and monitored under ICU-standards for 72 hours, after which they were sacrificed. The control group consisted of six animals. Two trauma treatment methods were applied, early total care (ETC, n=7), and damage control orthopaedics (DCO, n=8). Furthermore, a separate group (n=4) was subjected to ETC and treated with a C5-convertase inhibitor and anti-CD14. For this study, fracture hematoma, bone from the fracture site, bone from an unfractured long bone (humerus), and blood plasma were sampled. MicroRNAs were isolated, transcribed and pooled for qPCR array analysis.

The array data revealed distinct microRNA expression levels, specific to the trauma treatment method and the application of anti-complement/anti-CD14 medication. Overall, anti-inflammatory microRNAs were upregulated in the ETC group as compared to the DCO group. The ETC group that received anti-complement/anti-CD14 medication showed a reduced expression of several of these anti-inflammatory microRNAs. In all treatment groups, the expression levels of pro-fibrotic microRNAs were lower than the expression levels of anti-fibrotic microRNAs. A major difference was observed between the fracture site samples from the ETC

and DCO groups. MicroRNAs related to fibrosis were mostly downregulated in the DCO group, as compared to the upregulation of fibrotic microRNAs in the ETC group. Plasma microRNA expression revealed uniformly expressed circulating microRNAs, as well as multiple trauma specific microRNAs, and treatment specific microRNAs.

This study revealed microRNA expression profiles in fracture hematoma, bone, and plasma samples from a porcine multiple trauma model, linked to key processes in inflammation and fracture healing. Furthermore, the immunological response after multiple trauma seems to be represented in the systemic circulation through the expression of specific circulating microRNAs. Further research will focus on target analysis of the microRNA data, and in vitro fracture healing models in which mimics and antagomirs will be applied as possible regenerative and immunologic modulative therapy.

keywords: microRNAs, multiple trauma, complement system, fracture healing, bone regeneration

94238126164

IDENTIFICATION OF THE BEST MANUFACTURING CONDITION FOR CLINICAL GRADE EXTRACELLULAR VESICLES (EVS) SECRETED BY INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF OSTEOARTHRITIS

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Mesenchymal stem cells (MSCs) have been studied for the treatment of Osteoarthritis (OA), the most common chronic disease of joint cartilage. A potential mechanism of MSC-based therapies has been attributed to the paracrine secretion of trophic factors, where extracellular vesicles (EVs) may play a major role. It is suggested that MSCs from younger donor sources compete are optimal with respect their EV production capabilities. Therefore, MSCs generated from induced pluripotent mesenchymal stem cells (iMSCs) may represent a promising cellular source for the manufacture of EV therapeutics. In this study, we isolated and tested the efficacy of EVs secreted by MSCs and by iMSC for treatment of OA using an in vitro model.
To obtain high-quality EVs, we optimized the culture conditions for MSCs and iMSCs, the supernatant collection time, and EV extraction methods. MSCs and iMSCs were cultured in vitro in serum-free clinical grade condition. The cells were characterized for surface expression pattern, proliferation ability, senescence rate and differentiation capacity during long term-expansion. The culture media were collected continuously during the cell expansion, and EVs were isolated using an FPLC-anion exchange chromatography (AEX) approach. Nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blots as well as non-conventional flow cytometry were used to identify EVs. We evaluated the biological effects of MSC and iMSC-derived EVs on IL-1αtreated human chondrocytes, to mimic the OA environment.
We observed that the use of a serum-free, chemically defined medium for isolation and culture of hMSCs allowed us to expand a population with a stable phenotype from early to late passages. It is already well known that MSC proliferation, differentiation and function decline with passaging, in fact, after 3 passages we observed a drastic impact on cell growth and differentiation. Paracrine activity of hMSCs during long-term expansion was also evaluated. The number and size of vesicles released by hMSCs increased proportionally with their age in culture. EVs collected during hMSC long-term expansion retained tetraspanin (CD9, CD63 and CD81) expression and did not vary with parental cells age. Anti-inflammatory activity of MSC-EVs were evaluated in an in vitro model using osteoarthritic chondrocytes; administration of hMSC-EVs showed positive effects for early passages-derived vesicles only. The expression of IL-6 and IL-8 was significantly reduced after treatment with hMSC-derived EV at passage3. Over time in culture, the dimension of the vesicles increased while their anti-inflammatory effect was reduced. Concurrently, the expansion of iMSCs in serum-free conditions in vitro was optimized to define the best culture conditions to maintain the cells and to define the best time window in which to isolate EVs with maximum biological activity.
Despite the promising potential of EVs for therapeutic applications, robust manufacturing processes that would increase the consistency and scalability of EV production are still lacking. The focus of our study was directed on determining the optimal range of time in which MSCs and iMSC are biological functionally with respect to production of EVs in a serum-free culture

system. This paracrine application may represent a novel therapeutic approach for the treatment of OA.

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keywords: extracellular vesicles; induced pluripotent stem cell-derived mesenchymal stem cells; Mesenchymal stem cells; Osteoarthritis; Regenerative medicine

62825476779

A 3D MODEL FOR THE SURVIVAL NICHE OF HUMAN LONG-LIVED BONE MARROW PLASMA CELLS

Zehra Uyar-Aydin (Technische Universität Berlin, Berlin, Germany), Roland Lauster (Technische Universität Berlin, Berlin, Germany), Sina Bartfeld (Technische Universität Berlin, Berlin, Germany), Mark Rosowski (Technische Universität Berlin, Berlin, Germany)

Human long-lived plasma cells (LLPCs) are terminally differentiated effector cells of the B-lymphocyte lineage that reside in specialized niches in the human bone marrow (BM) harboring many different microenvironmental niches. LLPCs play an essential role in the humoral immune protection by maintaining constant high-affinity antibody levels against pathogens and their toxic products, independently of antigen presence. So far, the in vitro long-term cultivation of BM LLPCs is challenging since they reveal a brief survival time ex vivo. Thus, this project aims to develop an in vitro model that mimics the physiological microenvironment of their niche in the BM and enables long-term cultivation of LLPCs.

Our previously developed 3D model based on a hydroxyapatite-coated zirconium oxide-based ceramic can be used to maintain hematopoietic stem and progenitor cells for up to 8 weeks in their undifferentiated state (CD34+CD38-) when co-cultivated with BM mesenchymal stromal cells (MSCs) (Sieber et al. 2018). Based on this data, we aim to adapt the model to establish a microenvironment to support the survival of functional LLPCs in vitro. Human plasma cells (PCs) (CD38+CD138+) are isolated via magnetic activated cell sorting (MACS) from femoral head after mechanical preparation and introduced into the ceramic pre-seeded with MSCs building up a BM microenvironment. The survival capacity of functional PCs is assessed by flow cytometric analysis and detection of secreted antibodies by Bioplex.

So far, we are able to maintain PCs for up to 21 days in our in vitro system built up by the 3D scaffold pre-seeded with MSCs. Due to limitations of efficient extraction of PCs embedded in the 3D microenvironment after cultivation for flow cytometric analyses, their survival is shown indirectly by making use of their ability to secrete immunoglobulins. The cultured PCs remain functional and maintain their ability to secrete immunoglobulins (IgG1, IgA, IgM) over the culture time.

The established survival niche model could serve as a system to study niche interactions and will pave the way to establish disease models for diseases like multiple myeloma or autoimmunity to analyze changes in the microenvironment that promote the maintenance of pathogenic PCs. The better understanding of survival mechanisms of pathogenic PCs could disclose new targets for specific therapies.

keywords: bone marrow microenvironment, long-lived plasma cells

20941822305

OPTIMISING MRNA DELIVERY TO MESENCHYMAL STEM CELLS FOR TISSUE ENGINEERING APPLICATIONS

Katie McCormick (Royal College of Surgeons in Ireland, Dublin, Ireland), Jorge Moreno Herrero (BioNTech, SE, Mainz, Germany), Sarinj Fattah (Royal College of Surgeons in Ireland, Dublin, Ireland), Andreas Heise (Royal College of Surgeons in Ireland, Dublin, Ireland), Fergal O'Brien (Royal College of Surgeons in Ireland, Dublin, Ireland), Sally Ann Cryan (Royal College of Surgeons in Ireland, Dublin, Ireland)

INTRODUCTION: Within the field of tissue engineering, biomaterial scaffolds augmented with gene therapeutics have emerged as a promising treatment strategy for tissue regeneration. To date, the majority of 'gene-activated' scaffolds in tissue engineering have utilised plasmid DNA as the gene therapeutic of choice. Recently, messenger RNA (mRNA) has emerged as an attractive alternative to DNA-based therapeutics due to its increased safety profile and faster protein expression. The aim of this study is to optimise the delivery of mRNA to difficult-to-transfect mesenchymal stem cells (MSCs) and incorporate optimised particles into collagen scaffolds to create a platform that can be used for multiple tissue engineering (TE) applications.

METHODS: A wide range of non-viral gene delivery vectors were screened for their ability to encapsulate and condense mRNA. The complexes were characterised in terms of physicochemical properties before being brought forward to transfection studies using rat MSCs. All mRNA complexes were compared in terms of transgene (luciferase) expression and cytotoxicity in MSCs grown both in 2D monolayer and in 3D on collagen-based scaffolds. In addition, three different types of mRNA – unmodified mRNA (uRNA), modified mRNA (modRNA) and self-amplifying mRNA (saRNA) (BioNTech) were screened to determine the effect of mRNA type on expression for TE applications.

RESULTS: Various polymeric and lipid-based vectors were found capable of successfully delivering mRNA to MSCs. It was found that both the vector and mRNA type used had a significant impact on transgene expression in our cell type. Overall, the base-modified mRNA achieved the highest levels of protein expression in MSCs demonstrating a 1.2-fold and 5.6-fold increase versus uRNA and saRNA respectively in 2D monolayer studies. When delivered from a collagen-based scaffold, lipid-based vectors (e.g. MessegerMax/RNAiMAX) outperformed polymeric vectors (e.g. jetPEI) and achieved high levels of protein expression in the MSCs (2.3x106 relative light units).

CONCLUSION: Messenger RNA represents a promising tool for tissue engineering applications. This study highlights the optimised transfection conditions for mRNA delivery to mesenchymal stem cells in 2D and 3D. It is hoped that this work will serve as a template for future translational research within the field.

keywords: mRNA, gene therapy, tissue engineering

S35+S36
Giving meaning to early tissue damage responses in regeneration + Glycomodulation Approaches in Tissue Engineering
Room: S3 A
(1 Jul 2022, 11:00 - 12:30)

Conveners:
Johannes Grillari; Heinz Redl; Laura Russo;
Abhay Pandit

ENDOGENOUS BIOELECTRIC CONTROLS OF GROWTH AND FORM

Michael Levin (Distinguished Professor, Director of Allen Discovery Center at Tufts University)

Embryos and regenerating systems produce very complex, robust anatomical structures and stop growth and remodeling when those structures are complete. How do cell collectives know what to build and when to stop? Control of this process would provide a radical solution to injury, cancer, and perhaps aging. In this talk, I will discuss new tools and data that are revealing how groups of cells use bioelectric networks to coordinate the building and repair of organs. I will show examples of molecular modulation of bioelectric signaling in vivo that enables regeneration of limbs and correction of complex birth defects. I will also discuss computational tools that are now enabling us to design combinations of ion channel drugs (electroceuticals) as a roadmap to regenerative medicine.

52419502605

USING SUPRAMOLECULAR BIOMATERIALS TO INTERROGATE AND MANIPULATE GALECTIN-GLYCAN INTERACTIONS

Greg Hudalla (University of Florida, Gainesville, United States)

Covalent carbohydrate conjugates, referred to as glycans, decorate the surfaces of all mammalian cells and are found on nearly half of all mammalian proteins. Proteins that recognize specific glycan structures, known as lectins, play a central role in decoding the information stored within glycans and converting this information into signals that direct changes in cell behavior. Galectins are a specific subset of secreted lectins that can act as signaling molecules in healthy and pathological processes throughout all stages of life. Our research program employs supramolecular biomaterials as scaffolds to interrogate and manipulate galectin-glycan interactions. Supramolecular biomaterials are an ideal scaffold for these efforts because they allow us to mimic the multivalent architectures of galectins and glycans that are critical for their binding and biological activity. For example, we are developing beta-sheet peptide nanofibers modified with glycan appendages as synthetic glycoprotein mimetics. By tailoring glycan density or glycan chemistry on the nanofibers, we can tune their galectin binding affinity or specificity. We can use these glycopeptide nanofibers to disrupt or augment galectin signaling by changing the manner in which they are presented to cells (e.g. solution versus solid-phase). We also employ alpha-helical coiled-coils as scaffolds for multivalent galectin display. Using these scaffolds, we can create tools to study valency-function relationships of galectin-3. We can also use these scaffolds to create delivery vehicles that anchor a therapeutic enzyme at an injection site by endowing the enzyme with extracellular glycan-binding affinity. Finally, we can use coiled-coil scaffolds to create new anti-inflammatory therapeutics by recombining different galectins into non-natural multivalent architectures. Collectively, the examples presented in this talk will highlight the broad potential of supramolecular biomaterials to interrogate and manipulate galectin-glycan interactions, and identify new opportunities to exploit galectin-glycan interactions for tissue engineering.

keywords: protein-carbohydrate interactions, glycans, galectins, glycobiology

52354502655

ENHANCING TISSUE REGENERATION BY DELIVERING AN ENGINEERED TREG-DERIVED FACTOR

Celeste Piotto (Monash University, Melbourne, Australia), Ziad Julier (Monash University, Melbourne, Australia), Yen-zhen Lu (Monash University, Melbourne, Australia), Jacqueline Larouche (Monash University, Melbourne, Australia), Mikael Martino (Monash University, Melbourne, Australia)

Regulatory T cells (Tregs) are immuno-suppressive cells which have been recently rediscovered as pro-regenerative cells. When accumulating in injured tissue, Tregs express numerous pro-healing factors such as anti-inflammatory molecules and cytokines. Interestingly, we found that a growth factor (GF) is expressed by Tregs across multiple injured tissues in the mouse. Therefore, we hypothesized that delivering a recombinant form of the GF into damaged tissues would promote healing.

In order to optimize the activity of the GF following delivery in tissues, we first aimed at improving its signalling, utilizing rational protein engineering. As a strategy, we modified the receptor-binding site of the GF taking inspiration from the receptor-binding site of a high-affinity ligand of the same receptor, in order to generate a new high affinity variant (GFhi). The improved affinity of GFhi for the receptor was confirmed with various binding assays. A cell line was used to investigate the nature of the signalling through experiments of receptor internalization/degradation and phosphorylation. The regenerative capacity of GFhi compared to the wild-type GF was tested in mouse models of bone regeneration (calvarial defect), muscle regeneration (volumetric muscle loss of quadriceps) and skin repair (full-thickness wound). The GFs were delivered via a fibrin hydrogel in bone and muscle and via intradermal injections in the skin. To understand the mechanisms behind GFhi regenerative capacity, we tested its ability to affect proliferation of stem/progenitor cells which are important for the healing process such as keratinocytes, myoblasts, mesenchymal stem cells, and endothelial cells. We also investigated if GFhi could modulate macrophage activity, since they are key cells involved in tissue repair and regeneration. On this regard, we assessed GFhi regenerative potential in mice lacking its receptor on myeloid cells.

We show that GFhi has a higher binding affinity for its receptor compared to the wild-type GF. GFhi also shows a higher ability to induce receptor internalization/degradation and phosphorylation compared to the wild-type GF. In vivo experiments show that local delivery of GFhi promotes a more complete healing compared to the delivery of the wild-type GF in bone, muscle and skin models. Mechanistically, GFhi showed to have some proliferation effect on stem/progenitor cell. More interestingly, preliminary data where GFhi was delivered in mice lacking the GF receptor on myeloid cells indicates that the engineered GF likely promotes regeneration via modulating macrophage activity. Overall, this study shows that delivering a factor highly expressed by Tregs is able to induce tissue regeneration. Moreover, engineering the activity of key Treg-derived factors is a promising strategy for regenerative medicine applications.

keywords: Growth factors, Regulatory T cells, protein engineering, inflammation

41883627606

ELASTIN-LIKE-RECOMBINAMER CRYOGEL WITH RECOMBINANT GLYCOSAMINOGLYCANS AS A MODULAR PLATFORM FOR REGENERATION

Zackarias Söderlund (Lund University, Lund, Sweden), Linda Elowsson Rendin (Lund University, Lund, Sweden), Solmaz Hajizadeh (Lund University, Lund, Sweden), Emil Tykesson (Lund University, Lund, Sweden), Arturo Ibáñez-Fonseca (Lund University, Lund, Sweden), José Carlos Rodríguez-Cabello (Universidad de Valladolid, Valladolid, Spain), Lei Ye (Lund University, Lund, Sweden), Gunilla Westergren-Thorsson (Lund University, Lund, Sweden)

INTRODUCTION:
Multiple organs consists of macroporous structures such as the lung, bone, and kidney. However, when creating porous materials, added factors are quickly washed away, thus there is a need to bind them to the material.
Elastin-like Recombinamer (ELR) hydrogel is a type of biomaterial that has proved to have excellent biocompatible properties [2], where we here create a macroporous version using the cryogelation technique and functionalize it with recombinant glycosaminoglycans to have a controlled release of growth factors.

METHODS:
ELR is a two-part solution, which are modified so that one part has an alkyne modification while the second part has an azide modification. When these two parts are mixed a covalent bond is formed resulting in a hydrogel. ELR cryogel was created by forming the hydrogel in subzero temperatures. Azide containing glycosaminoglycans were covalently added during the formation to slow the release of growth factors.

RESULTS:
ELR cryogels were subcutaneously implanted in mice, where cryogels with added growth factor only showed an increased blood vessel formation but the addition of recombinant glycosaminoglycans in combination with growth factor showed a change in the immune response going from a more inflammatory state to a more regenerative state shown by the shift of present macrophage phenotypes.

CONCLUSION:
The data showed that an ELR-based cryogel is a promising synthetic scaffold for tissue engineering, mimicking the 3D environment of the extracellular matrix and that recombinant glycosaminoglycans can be added for a controlled release of growth factors.

ACKNOWLEDGMENTS:
Financial support was received from the Swedish Heart-Lung Foundation, Swedish Research Council, Crafoord Foundation, Royal Physiographical Society of Lund, Österlund Foundation.

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keywords: Cryogel, Glycosaminoglycans , Elastin-like Recombinamer

20941838684

GUIDED BONE REGENERATION IN OSTEOPOROSIS BY PLANT-DERIVED NANOPARTICLES

Katarzyna Gurzawska-Comis (University of Liverpool, Liverpool, United Kingdom), Salwa Suliman (University of Bergen, Bergen, Norway), Anna Mieszkowska (Jagiellonian University, Krakow, Poland), Samih Mohamed-Ahmed (University of Bergen, Bergen, Norway), Anna Finne Wistrand (KTH, Royal Institute of Technology, Stockholm, Sweden), Bodil Jørgensen (University of Copenhagen, Copenhagen, Denmark), Kamal Mustafa (University of Bergen, Bergen, Norway)

Background:
The repair and treatment of large bone defects in patients with compromised bone metabolism due to ageing and medical conditions such as osteoporosis present often a clinical challenge. Therefore adjunctive methods to enhance bone healing are needed. Bone tissue engineering with application of nanotechnology allows to construct biomaterials with desired properties being osteoconductive, osteoinductive and osteogenic.

Aim/Hypothesis :
The aim of our study was to promote bone regeneration using functionalised scaffold with Rhamnogalacturonan-I pectins (RG-I) in vitro and in vivo using aging and osteoporotic rodent models.

Material and Methods :
The biomaterials were poly(l-lactide-co-ε-caprolactone) scaffolds and the RG-I was from potato. The chemical and physical properties of functionalised biomaterials with RG-I nanoparticles were characterised using confocal and atomic force microscopy. Functionalised scaffolds with RG-I (tested sample) were evaluated in vitro with human osteoblasts from osteoporotic patients and their response was tested using real-time PCR. In vivo evaluation was performed using criticalsize calvaria bone defect model in ageing and osteoporotic rat models. Scaffolds were implanted randomly in the calvaria defects of aged female Wistar rats (11-12 months old) and osteoporotic female Wistar rats induced by ovariectomy. The control was scaffold without RG-I. After 2 and 8 weeks animals were euthanised. Harvested samples were analysed for osteogenic and inflammatory markers using real-time PCR. Bone formation was evaluated radiographically and histologically. The data was analysed using one-way ANOVA.

Results:
The chemical and physical properties results indicated success of the functionalisation of scaffolds with RG-I. Osteoblasts response suggested osteogenic (upregulation osteopontin, osteocalcin, collagen1, bone sialoprotein) and anti-inflammatory properties (downregulation IL-1, IL-8, TNFalpha) on the scaffold functionalised with RG-I. The in vivo results in aged and osteoporotic rat calvaria model of early (2 weeks) bone regeneration showed increase of osteogenic markers and decrease of proinflammatory markers and RANKL, compared to control. In osteoporotic rat model at week 2 and 8 and in aged rat model at week 8, the mean percentage of BV/TV (bone volume/tissue volume) in the defect with RG-I scaffold was significantly greater than the defect with control. The histological evaluation in both rat models revealed larger areas of new bone formation in RG-I scaffolds than in control.

Conclusion and Clinical implications :
In conclusion, the plant-derived nanoparticles significantly increased osteogenic and decreased pro-inflammatory response in vitro and in vivo. These finding may have a crucial impact on bone repair process especially in elderly and osteoporotic patients.

keywords: Osteoporosis, Pectin, Bone regeneration, Scaffold, Nanotechnology

S37
Human Organoids for Musculoskeletal Tissues
Room: S4 A
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Debby Gawlitta; Xiao-hua Qin

52419506939

ENGINEERING GRAFTS FOR JOINT REGENERATION USING PHENOTYPICALLY DISTINCT CARTILAGINOUS MICROTISSUES

Daniel Kelly (Trinity College Dublin, Ireland)

Traditionally, tissue engineering strategies employ a "top-down" approach, where cells are randomly seeded in polymeric scaffolds or hydrogels. As a result, engineered tissues are often at best homogenous in composition, lacking the morphological or structural features of native tissues. Alternative "bottom-up" approaches, that leverage the self-organizing capacity of stem cells, have shown promise for engineering human tissues [1]. During early limb development stem cells aggregate and condensate before differentiating along a chondrogenic lineage.
In fact, pellet culture, where mesenchymal stem cells (MSCs) are forced to aggregate using centrifugation, has been the standard culture system for initiation of stem cell chondrogenesis in vitro as it allows cell-cell interactions that are analogous to those that occur during pre-cartilage condensation during early joint development [2]. Therefore cellular aggregates, microtissues or organoids might represent promising biological building blocks for the engineering of functional tissues.
This talk will describe how phenotypically distinct microtissues generated from stem/stromal cells can be integrated to engineer a biphasic osteochondral implant containing a biomimetic layer of engineered articular cartilage. The osseous region of this osteochondral graft was engineered using islands of hypertrophic cartilage microtissues capable of executing an endochondral programme, while the chondral region of the graft was formed by the self-organisation of early-cartilage microtissues into a unified and structurally organised tissue mimetic of native AC. Furthermore this talk will examine whether implantation of such an engineered plug into critically-sized caprine osteochondral defects can result in effective biological joint resurfacing and prevent the deleterious cascade of events that typically follow an untreated osteochondral injury.

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keywords: microtissue, aggregate, cartilage, osteochondral, biofabrication

31451702964

STRUCTURAL SUPPORT FOR HUMAN CARTILAGE ORGANOIDS

Jos Malda (UMC Utrecht, Utrecht, Netherlands)

Organoids (self-assembled 3D tissue structures from a cluster of cells) can be used for patient-specific drug testing or for the generation of larger tissue constructs, which can in turn be used as implants to restore and/or replace damaged tissue. For cartilage, human chondrocytes, both healthy and diseased (derived from patients with osteoarthritis (OA)), show the potential to self-assemble into organoid structures. However, these organoids do not show the distinct collagen architecture that is needed to withstand the loading conditions in joints. Recently, the convergence of self-assembled equine articular cartilage-derived progenitor cells (eACPCs), which were stimulated with BMP-9 to increase the rate of matrix formation, with micrometer-scale reinforcing PCL fibres (made with melt electrowriting (MEW)) shows the potential to create abundant cartilage-like tissue while providing a mechanical support structure that is capable of withstanding in vivo loading conditions. We envision that the convergence of reinforcing technologies, such as MEW, with the human OA-derived organoids allows us to tackle the challenges of the availability of cell sources and limited mechanical properties of implants and with that steers towards patient-specific implants.

keywords: Melt electrowriting, Cartilage, BMP-9, Bioprinting

52354555506

MICROENGINEERED 3D BONE CELL MODELS VIA IMAGE-GUIDED TWO-PHOTON SUBTRACTIVE LITHOGRAPHY

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Despite immense interests, growing organoids resembling the human musculoskeletal system (including bone, cartilage, muscle, tendon, ligament) in a petri dish remains a major challenge in front of the tissue engineering and regenerative medicine (TERM) community. Bone is a vital organ that contains billions of bone cells as well as a sophisticated internal architecture across several length scales. Recapitulating the structural and functional complexity in bone requires the development of high resolution biofabrication techniques that faithfully recreate tissue architecture down to the micrometer-scale accuracy. One promising approach is to combine computer models derived from biomedical imaging data with light-based tissue manufacturing.

Here, we present an image-based subtractive biomanufacturing process to create microengineered 3D bone cell models in biocompatible hydrogels. To this end, new computer models that mimic the topology of lacuno-canalicular network (LCN) in bone are developed by sequential immunostaining and confocal microscopic imaging of osteocytes in bone specimen. These models are converted into stereolithography (.STL) files through image processing. Using two-photon subtractive lithography, we demonstrate the fabrication of LCN-mimicking microstructures inside a photodegradable polyethylene glycol hydrogel at high spatial resolution. The structural fidelity is highly dependent on the laser processing parameters such as laser power, writing speed and photosensitivity of the hydrogel matrices. The inclusion of a soluble two-photon photoinitiator can greatly decrease the laser threshold. Lastly, preliminary success on biomimetic subtractive 3D microprinting in the presence of living bone cells and guided 3D cell growth will be presented towards a living bone organoid.

keywords: Musculoskeletal, bone, organoids, microengineering, biofabrication

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INCREASED CELL DENSITY INCREASES MINERAL FORMATION RATES AND STIFFNESS IN 3D BIOPRINTED PATIENT-DERIVED BONE ORGANOIDS USING DYNAMIC LOADING

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Bone organoids are an emerging novel platform to study human bone biology and bone formation. Biofabrication techniques such as extrusion bioprinting have been used to produce mineralized in vitro bone models. However, cell-cell interactions and mineralization rates are influenced by the initial cell printing density [1]. Here, we investigated the effect of cell density on mineral formation, organoid stiffness, and cell morphology in human 3D bioprinted cell-laden hydrogels under dynamic culture conditions.

Osteoblasts isolated from a femur bone chip of a 15-year-old male were encapsulated in 0.8% (w/v) alginate, 4.1% (w/v) gelatin, 0.1% (w/v) graphene oxide hydrogels [1]. Cells were extrusion bioprinted at 5x106 or 10x106 cells/mL of hydrogel and cultured in compression bioreactors for 10 weeks. Organoids were subjected to uniaxial cyclic compressive loading with 1% strain at 5Hz for 5 minutes 5 times per week. Live/dead assays were performed to determine cell viability after bioprinting (day 1) and after two weeks (day 15) of mechanical loading. F-actin nuclear staining was performed after 30 and 70 days of loading to investigate cell spreading morphology. Time lapsed micro-computed tomography (micro-CT) scans were taken weekly to monitor mineral volume and density. After 10 weeks of loading, organoids were fixed and cryosectioned for histology, immunohistochemistry, and scanning electron microscopy (SEM) to evaluate cellular phenotypes and mineralized matrix formation.

Patient-derived organoids exhibited high cell viabilities after bioprinting (>90%) and after two weeks of daily mechanical loading (>85%). F-actin staining after 30 and 70 days, revealed increased cell spreading and dendrite number in higher cell density organoids. While time lapsed micro-CT images revealed similar endpoint mineral volumes, significant differences were found when comparing mineralization rates and mineral densities between the two cell density groups. Higher cell density organoids exhibited the highest mineral formation rates in the earlier timepoints (28-35 days) while lower cell density organoids reached peak mineral formation after 49-56 days. Notably, a significantly higher average mineral density of 230.8 ± 15 mg HA/cm3 was found in higher cell density organoids compared to 176.9 ± 21.42 mg HA/cm3 in low cell density organoids after 70 days of culture. In line, higher cell density organoids exhibited increased stiffness as compared to lower cell density organoids. A 10-fold increase in stiffness was observed in higher cell density organoids at endpoint compared to day 15 measurements. Meanwhile, lower cell density organoids only showed a 2-fold increase in stiffness during this time. Histology, immunohistochemistry, and SEM imaging revealed distinct cell morphologies in the organoids, including osteoblastic and osteocytic characteristics.

Here, we have established a methodology that better supports the formation of mineralized patient-derived bone organoids resembling native bone tissue. Bioprinting at higher cell densities increased organoid stiffness and mineral density. These clinically relevant bone organoids combine primary patient cells with physiological loading conditions to study mineral formation of healthy bone. In future studies, this platform will be employed to investigate pathological bone and evaluate potential therapies.

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keywords: bone organoid, 3D bioprinting, mineralization

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DIRECTING HUMAN MESENCHYMAL STEM CELLS DIFFERENTIATION TOWARDS HYPERTROPHIC CHONDROCYTES USING FIBER-REINFORCED BONE DECM HYDROGEL SCAFFOLDS

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Intramembranous ossification is the most common pathway used in tissue engineering (TE) of bone. However, therapeutic effects in case of large bone defects are suboptimal due to hindered vascularization. On the other hand, TE grafts generated via the endochondral ossification pathway, where the bone formation occurs through cartilage intermediaries, become vascularized and form bone upon implantation. In order to build bone, the chondrocytes have to become hypertrophic. The goal of this study was to develop fiber-reinforced bone decellularized extracellular matrix (bdECM)-based hydrogels for enhanced hypertrophic differentiation of chondrogenicly primed human bone marrow MSC (hMSC).

The hydrogels were composed of either 10 mg/ml type I collagen or bdECM mixed with alginate (30 mg/ml). As the reinforcement, 3D fibrous scaffolds were fabricated using PCL and PCL-based composites containing 25 wt% of β-TCP and FDM technology. HMSC were encapsulated in the pre-gel at density of 20x106/ml, infused into the 3D scaffolds (50 μl of cell suspension/construct) and cultured in chondrogenic medium for 28 days. To measure cell viability, live/dead staining and MTS assay were performed. Secretion of cartilaginous matrix was evaluated by staining for safranin O, collagen type I, II and X. Moreover, quantification of GAGs was performed using 1,9-dimethylmethylene blue assay and collagen using total collagen assay. Additionally, alkaline phosphatase (ALP) activity was measured using colorimetric assay with para-nitrophenyl phosphate as a substrate.

The cell metabolic activity did not decrease upon culture, although the overall viability measured by live/dead staining decreased, suggesting cell proliferation. A synergistic effect of the dECM and β-TCP on hypertrophic differentiation of hMSC was measured my means increased ALP activity and visualized by more intense staining against type X collagen. The aforementioned finding suggests that bone dECM hydrogels reinforced with β-TCP/PCL scaffolds are suitable materials for chondrogenic priming of MSC and bone TE via osteochondral pathway.

keywords: decellularized ECM, mesenchymal stem cells, hypertrophic chondrocytes, endochondral ossification, bone tissue engineering

52354526488

THE INTERPLAY BETWEEN IMMUNE RESPONSE AND BONE FORMATION FROM DEVITALIZED ALLOGENEIC CELLS

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Introduction
Autologous bone grafting is currently the gold standard treatment for bone defects. However, it is still associated with multiple drawbacks such as limited availability and donor site morbidity. Various approaches have been explored to overcome this, of which endochondral bone regeneration (EBR) has emerged as a promising approach. EBR aims to mimic the process where bone is remodeled from a cartilage template, which naturally takes place in a fracture callus. Clinical translation of EBR would benefit from creating an off-the-shelf product consisting of donor-derived (allogeneic) and devitalized tissues. We have previously shown that such allogeneic devitalized cartilage tissues display accelerated full-bridging of a femoral defect in immunocompetent rats [1]. However, there is still a need to fully elucidate the interplay between the immune response and new bone formation. To explore this further, the early and late immune response elicited by vital and devitalized cartilage derived from autologous or allogeneic cells were compared in a subcutaneous rat model.

Methods
Rat multipotent mesenchymal stromal cells (rMSCs) were isolated from the bone marrow of Brown Norway (BN; autologous/syngeneic) and Dark Agouti (DA:allogeneic) rats. rMSCs were encapsulated within collagen hydrogel spheroids and chondrogenically differentiated for 28 days, followed by devitalization [1]. Two chondrogenic spheroids per group were implanted into subcutaneous pockets in BN rats (n=7) for 3,7,14,28 and 84 days. New bone formation and immune response were analyzed via micro-CT, histology (H&E, histomorphometry) and immunohistological staining (CD68,iNOS,CD206,CD3,myeloperoxidase).

Results and Discussion
Histological analyses revealed that the vital autologous spheroids were remodeled into bone including marrow cavities within 28 days. At this time, remnants of non-remodeled cartilage were still observed for the vital allogeneic and both devitalized groups. After 84 days, 5/7 samples from the vital autologous group could not be retrieved possibly due to resorption. Histomorphometry analyses from day 28 and 84 revealed no significant differences in bone formation or cartilage between groups (excluding vital autologous due to reduced sample size). The onset of bone formation appears to positively correlate with the presence of osteoclasts on day 14 in the vital autologous and both devitalized groups. Presence of osteoclasts in the vital autologous and both devitalized groups on day 84 indicate that active remodeling is still taking place. Initial analyses of the immune response revealed no significant differences between groups in terms of presence of macrophages (CD68, CD206 or iNOS) or T lymphocytes in the tissue surrounding the implants on day 3, 7 or 14. Further analysis of later timepoints and

different immune cells are still on-going.

Conclusion
No differences in bone formation was observed between all groups. However, the vital autologous group demonstrated the fastest bone formation, most of which were resorbed by 84 days. Early analysis indicate that the onset of bone formation coincides with the presence of osteoclasts from as early as 14 days. Additional analyses are currently ongoing to elucidate the interplay of immune cells and bone formation at later time points.

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keywords: allogeneic, immune response, devitalization, endochondral bone tissue regeneration, mesenchymal stromal cells

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TOWARDS BONE-REMODELING-ON-A-CHIP: FORMATION OF 3D BONE-LIKE TISSUES

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Introduction: Bone remodeling is the combined process of bone resorption by osteoclasts and bone formation by osteoblasts. This process is regulated by mechanosensing osteocytes. It is the most fundamental physiological process that defines living bone. An imbalance in this process can cause metabolic bone diseases such as osteoporosis. Currently, no complete in vitro bone remodeling model is available. Such models have the potential to increase our knowledge on the physiological and pathological processes underlying bone remodeling and could potentially improve drug development processes. Bone-on-a-chip technology has the great potential to advance bone research, allowing for the study of low cell numbers in high temporal and/or spatial resolution. In this study, microfluidic chip technology is used to create a bone remodeling model. Currently, the study focusses on three-dimensional (3D) bone formation inside the microfluidic chip by osteogenic differentiation of human bone marrow derived mesenchymal stromal cells (MSCs) into osteoblasts. Next, the aim is to achieve bone-remodeling-on-a-chip by facilitating interaction between osteoblasts, osteocytes and osteoclasts.

Methods: A bone-on-a-chip microfluidic device that facilitates 3D in vitro bone-like tissue formation was developed. A polydimethylsiloxane (PDMS) microfluidic device was fabricated by means of photo- and soft-lithography. The device contained rectangular-shaped cell culture channels that were coated with fibronectin and seeded with MSCs. The MSCs were dynamically cultured for a period of 21 days by applying medium flow, resulting in shear stresses of around 2.3 mPa acting on the cells. Osteogenic medium was used to differentiate the MSCs along the osteogenic lineage.

Results: Time-lapse brightfield imaging revealed self-assembly into 3D constructs within the channel. At the end of the 21-day culture period, deposition of calcium (Alizarin Red staining) and collagen (Picrosirius Red staining) in the extracellular matrix produced by the cells was visible. Confocal microscopy revealed the formation of 3D bone-like struts through self-assembly. Immunohistochemical staining confirmed the formation of collagen type 1 and revealed the expression of osteopontin and DMP-1, confirming the differentiation of the MSCs into the osteogenic lineage.

Conclusion: Overall, the results revealed mineralized bone-like struts. With this, the developed bone-on-a-chip microfluidic device showed the first step towards a 3D in vitro bone remodeling model, exhibiting 3D bone-like tissue formation. In future research, osteoclasts will be added to the model to facilitate the bone resorption process.

keywords: bone remodeling, in vitro models, bone-on-a-chip

S38
Injectable biomaterials for cell-instructive matrix cues
Room: S3 B
(29 Jun 2022, 13:30 - 15:00)

Conveners:
Mirosława El Fray

FIREFLY-INSPIRED BIOMATERIALS AS TUNABLE, TRIGGERABLE, AND CELL-INSTRUCTIVE MATRICES FOR 3D CELL ENCAPSULATION

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Introduction
Minimally invasive surgery for the restoration of bone tissues lost due to diseases and trauma is preferred to reduce patient complications and health care costs. The current challenge is to design material at the site of surgery with specific behaviors for mimicking the natural structures and delivering appropriate signals to cells promoting tissue repair/regeneration. Selection of a suitable injectable is often based on material characteristics (including mechanical properties, drug release kinetics and degradation) that serve for the specific treatment function. Micro or nano-structured materials in the form of gels, nanoparticles and nano-composites have gained increasing interest in regenerative medicine because they are able to mimic the physical features of natural extracellular matrix (ECM) at the sub-micro and nano-scale levels. Different strategies are implemented for engineering bioactive and osteoinductive injectable materials to optimize their interfaces with cells and bone tissue environments.
Here, it discussed injectable bone materials integrating biphasic calcium phosphate nanoparticles prepared by sol-gel synthesis with different polymers, nano-materials and antimicrobial compounds for specific bone infections conditions.

Methodology
Innovative natural polymer-based double network hydrogels (DNs) were developed by a two-step network-formation procedure to obtain photocrosslinkable methacrylated hyaluronic acid (HAMA) and maleated hyaluronic acid (MAHA). Different chemical modification of hyaluronic acid (HA) followed by the development of nanocomposites hydrogels were directly prepared in situ by sol-gel synthesis [1]. Furthermore, injectable hybrid material based on graphene oxide nanosheets and hydroxyapatite prepared by sol-gel approach is described. The presence of GO increases the bioactive and osteogenic material properties.
One more approach is based on the use of antimicrobial injectable materials [2]. Several systems based on Ionic Liquids (IL) at different alkyl-chain length incorporated in Hydroxyapatite through the sol-gel process were developed to obtain an injectable material with simultaneous opposite responses toward osteoblasts and microbial proliferation.
In vitro cell tests to assess the osteogenic potential of the synthesized biomaterials were performed using human mesenchymal stem cells (hMSC) and the expression of specific osteogenic markers (ALP, OCN) was analyzed.

Results
Nanocomposite materials based on chemically modified HAs and in situ sol-gel CaP, were successfully developed and characterized in terms of physico-chemical, morphological, mechanical and biological properties. Injectable bone materials integrating BCP nanoparticles with HA based materials, GO and IL were successfully synthesized. For HA-GO system, it was found that the spindle-like hydroxyapatite nanoparticles were intercalated between GO nanosheets. The oxygen-containing functional groups of GO sheets play an important role in anchoring calcium ions, as demonstrated by FTIR and TEM investigations, thus improving the bioactive and osteogenic properties. The systems based on CaP-ILs showed a higher osteogenic activity and antimicrobial performance by increasing the IL alkyl chain. These systems are able to induce osteogenic differentiation and also inhibit biofilm formation.

Conclusion
The results indicated that all the proposed injectable materials can be considered a high-performance bone filler in the treatment of bone defects

IN SITU ASSEMBLING BIOHYBRID POLYMER HYDROGELS TO MODULATE CELL-INSTRUCTIVE MATRIX CUES

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Glycosaminoglycans (GAGs) within extracellular matrices (ECM) control the presentation of soluble, cell-instructive signals. The incorporation of GAGs into engineered polymer networks can therefore provide a powerful and versatile means of directing cell fate. We have established a rational design strategy for ECM-inspired hydrogels based on multi-armed poly(ethylene glycol), GAGs of different sulfation patterns, and functional peptides to systematically explore the related options. Micro-processing schemes (cyrogelation, solvent-assisted micro-molding, microfluidic microgel fabrication, multicomponent inkjet bioprinting) allow for the fabrication of multiphasic and multifunctional GAG-based gel materials with spatiotemporally adjusted signaling characteristics. Applications of the materials platform include 3D culture models of stem cell and tumor microenvironments as well as scaffolds for exploring new therapeutic approaches to chronic wounds and neurodegenerative diseases.

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FIREFLY-INSPIRED BIOMATERIALS AS TUNABLE, TRIGGERABLE, AND CELL-INSTRUCTIVE MATRICES FOR 3D CELL ENCAPSULATION

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Introduction. One challenge of developing biomaterials for tissue engineering is the capability to precisely engineer desired properties, while keeping robustness and versatility in the system. Cell-encapsulating hydrogels are used as extracellular matrix mimics for basic study of cell function, high-throughput drug screening, and therapeutic delivery. In their molecular design, the crosslinking chemistry plays a vital role in regulating important properties, such as gelation rate, mechanical strength, and bioactivity [1]. Despite the many covalent crosslinking strategies reported so far, they are often not economic, user-friendly, or tunable enough to facilitate the adaptability of the encapsulating system to a variety of biomedical scenarios. To overcome this challenge, and inspired by the biochemistry of fireflies, we present a bioinspired covalent chemistry for the fabrication of precisely tunable, inexpensive, and versatile polyethylene glycol (PEG) hydrogels for 3D cell culture [2]. It is based on the condensation reaction between cyanobenzothiazole and cysteine groups, known as “luciferin click ligation”.

Methodology. 4-arm, 20-kDa PEG macromers bearing cyanobenzothiazole or cysteine functional groups were synthesized. Hydrogels were prepared under physiological conditions (37°C, HEPES buffer, pH 7-8). Crosslinking process, biofunctionalization with enzymatically cleavable and cell-adhesive peptides, and encapsulation of human mesenchymal stem cells (hMSCs) took place one-pot. The resulting hydrogels were cultured for 1-3 days. Cell viability, cell behavior and cell-materials interactions were evaluated by live/dead assay, F-actin cytoskeletal and morphological characteristics of cell analyses, respectively. Cell proliferation ability was assessed by Ki67+ nuclei staining. Mechanical strength and gelation kinetics of hydrogels were characterized by shear rheology.

Results. PEG hydrogels showed efficient and pH-regulable gelation rate, adjustable mechanical strength within physiologically relevant values, and high materials homogeneity at the microscale. By incorporating biochemical cues (i.e., cell-adhesive and cell-degradable ligands) to the hydrogel network, cell behavior and cell-materials interactions were modulated. Our gels supported the culture of hMSCs: encapsulated cells showed high cell viability (demonstrating the good cytocompatibility of these gels) and maintained their proliferation capability. 3D cell spreading (volume expansion), accompanied by high degree of cell protrusion and F-actin stress fiber formation, was observed in the presence of both cell-adhesive and cell-degradable cues in the gels.
To further develop the firefly-inspired hydrogel system as an injectable platform, novel redox-triggerable hydrogel precursors were introduced to the molecular design. The cysteine-based precursor was modified with a protecting group at the thiol residue, thus blocking gel crosslinking. Upon addition of a biocompatible reductant, the cysteine group was deprotected and the crosslinking reaction was triggered with exquisite control of the reaction rate. The storage stability of precursors was also improved, which is convenient for future upscaling and translation [3].

Conclusions. Firefly-inspired gels are robust and provide versatility for easy adaptation to diverse biomedical situations. Molecular engineering confers higher user control for the

fabrication of injectable biomaterials. These biomaterials are expected to become valuable platforms for tissue engineering.

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keywords: bioinspired materials, tunable properties, injectable hydrogels, cell-materials interactions, biological properties

94238166968

DEVELOPMENT OF IN SITU CROSSLINKABLE BIORESPONSIVE ALGINATE HYDROGELS

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Introduction
Alginate is widely used in the biomedical field, particularly to build three-dimensional (3D) systems with ECM-like properties. Despite being a bio-inert biomaterial, alginate can be chemically modified to promote highly specific cell-ECM interactions[1]. We have shown that molecularly designed alginate 3D matrices recap key features of native ECMs supporting tissue morphogenesis[2-4]. Here, we developed a novel methodology to synthesize and characterize functional bioresponsive alginate hydrogels based on thiol–maleimide “click” chemistry. The potential of these peptides-conjugated hydrogels as ECM-like matrices for 3D cell culture was evaluated.

Methodology
Ultra-pure low viscosity alginate (Alg) with high guluronic content was modified with variable amounts of maleimide (AlgM) by amidation of alginate carboxyl groups with the amine groups of 1-(2-Aminoethyl)-maleimide. Degree of substitution was assessed by 1H-NMR. Thiol-flanked (bi-functional) protease-sensitive peptide (GIW-peptide, CGPQGIWGQC) and thiol-terminated cell-adhesion peptide (RGD-peptide, CGGGGRGDSP) were grafted to AlgM via thiol-maleimide Michael addition click reaction. To confirm peptide double-end grafting and consequent crosslinking, Alg solutions dynamic viscosity was analyzed by oscillation/viscometry rheology. Alterations on GIW-crosslinked AlgM viscosity and molecular weight were assessed by gel permeation chromatography (GPC). Grafting of RGD-peptide was quantified by BCA Protein Assay (Pierce). Primary human mammary normal (nFIB) and cancer-associated (CAF) fibroblasts mixed with alginate and peptide solutions were cast as hydrogels. 3D cell response to different GIW/RGD-peptide concentrations was studied at different timepoints. MMP production (gelatin zymography), cell viability (live-dead assay), morphology (F-actin staining), and mechanical properties were assessed.

Results
1H-NMR qualitatively confirmed successful alginate functionalization with different maleimide amounts (theoretical substitution degree from 1 to 10%). Maleimide presence was identified by the appearance of a new peak (~6.9ppm) corresponding to the protons in the double bond of the maleimide group. Reaction efficiency was approximately 10%. We observed that high degrees of maleimides lead to poor solubility of alginate derivatives, so we only used derivatives with up to 0.3% of modification. Addition of bi-functional GIW-peptide increased hydrogel viscosity due to the formation of a chemically crosslinked gel network. Viscosity increase and consequent gel formation was observed between 120-480µM of GIW-peptide. Higher concentrations (840µM) did not alter solution viscosity but increased alginate Mw, because GIW-peptides were preferentially bound only by one side, occupying the maleimides without bridging two alginate chains. nFIB and CAF (fibroblasts with different proliferative/metabolic profiles) were successfully embedded within GIW/RGD-AlgM matrices, presenting elongated morphologies and forming extensive multicellular networks, contrary to control MMP-insensitive hydrogels, where cells remained essentially round. Due to its nature, CAF formed more extensive cellular networks faster, without major differences regarding MMP production.

Conclusions
A novel methodology for the synthesis of alginate containing maleimide functional groups was established. Covalently grafted maleimides allow alginate biofunctionalization and in situ crosslinking by thiol-Michael addition reaction. Incorporating protease-sensitive peptides significantly enhanced 3D cell-cell interactions in alginate hydrogels, improving their performance as ECM-mimics.

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keywords: 3D microenvironment, bioresponsive hydrogel, matrix metalloproteinase, alginate, click chemistry

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INJECTABLE NANOFIBROUS MICROSCAFFOLDS FOR CELL AND DRUG DELIVERY

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Injectable biomaterials for cell and drug delivery is a rapidly expanding field that may revolutionize medical therapies. Here we report on the fabrication of injectable electrospun microscaffolds used to deliver desired cargo through the needle. We observed an efficient attachment of cells to the scaffold's surface, creating cell-populated microscaffolds (MS) that could be injected or 3D printed through 23-26G needles.

Polymer nano- and microfibers were electrospun and then structured with a picosecond laser. To increase the hydrophilicity of the MS, nanofibers were first hydrolyzed with sodium hydroxide and then functionalized with natural polymers like chitosan or chondroitin sulfate. For morphological characterization of MS with and without cells, we used Scanning Electron Microscopy (SEM). Physico-chemical characterization was done to analyze the impact of laser processing on polymer nanofibers. We used the L929 cell line to assess the biocompatibility of produced MS and the possibility of injecting the construct into the desired tissue. Nucleus pulposus cells were used as the target cells to evaluate their survival after injection through different needle sizes.

The direct injection of cells into tissues faces several challenges, such as low survival and their retention at the injection site. With cell-protective MS, the survival rate can be significantly increased. When using laser processing, any shape of microscaffolds can be created, among others, MS with quadrilateral, triangular or circular base. Moreover, low melting of the fibres at the cut surfaces can be observed. The cytocompatibility assays show an increase in cell number with culture time. L929 cells populated MS at each side, resulting in the formation of agglomerates. The injectability studies through 24G and 23G needles showed that the ejection rate was 97% and 98%, respectively.

We developed a novel and straightforward method to fabricate microscaffolds from almost any type of electrospun material. MSs are compatible with living tissues and readily populated with cells. By designing the surface chemistry of nanofibers, the physical and chemical structure of MSs can be customized to improve cell-MS interaction. The injectability studies show that PLLA-based MSs are injectable through the tested range of needle sizes and could be well suited for minimally invasive cell delivery applications. One of the examined applications is intervertebral disc degeneration, where designed MS delivers active molecules that enhance the synthesis of glycosaminoglycans. A single administration of the drug in the MS to the tissue will result in several weeks of the release of the active substance, which may have a beneficial effect on the regenerative processes of the intervertebral disc.

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keywords: cell therapies, injectable scaffold, drug delivery, nanofibers, electrospinning, IVD

62825474529

CLICKABLE AMPHIPHILE ALGINATE PRODUCES DYNAMIC 3D CELL MICROENVIRONMENTS WITH MICROSTRUCTURED HYDROPHOBIC DOMAINS

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The components of cellular microenvironments, especially the extracellular matrix (ECM), strongly regulate biological processes through biochemical and mechanical signaling. For this, hydrogels have been widely used to build artificial niches mimicking the native ECM. Strain-promoted azide-alkyne cycloaddition (SPAAC) is a biorthogonal reaction between strained cyclic ring-containing alkynes and azides, which proceeds under mild, copper-free, biocompatible conditions. Here, we developed an amphiphile SPAAC-clickable alginate derivative capable of forming ionic hydrogels with hydrophobic microdomains, which can be further functionalized in-situ and in the presence of cells. This system provides topographical cues to cells within a true 3D microenvironment and may sequester molecules with hydrophobic moieties while allowing on-demand dynamic switch of matrix properties.

Ultra-pure alginate was functionalized with a cyclooctyne (BCN-amine) by carbodiimide chemistry. Modification degrees of alkyne-alginates (ALK) were assessed by 1H-NMR. ALK derivatives were characterized by contact angle measurements, hydrophobic probes, scanning electron cryomicroscopy, and viscometry. ALK hydrogels (acellular and cell-laden) were prepared by ionic crosslinking with Ca^{2+} [4]. Mechanical analysis was performed by microindentation and rheometry. SPAAC conjugation with azide-functionalized compounds was performed at 37°C, in pre-gel solutions (0.9% w/v NaCl) or pre-formed hydrogels (culture medium). Grafting kinetics and efficiency were estimated using fluorescent azido-tags. Clickable hydrogels laden with mesenchymal stem cells (MSC) were analyzed in metabolic activity and morphology, ECM protein expression by immunostaining, and gene expression for 14-d.

ALK with varying modification degrees was successfully produced. Increased modification produced ALK derivatives with less hydrophilicity, stronger interactions with hydrophobic probes, and increased viscosity in aqueous solutions. At higher modification degrees, ALK derivatives showed the ability to spontaneously establish concentration-dependent associations between polymer chains. Ionic ALK hydrogels with denser microstructures within a sparser 3D network were produced, showing spatial heterogeneity in stiffness, as expected. These regions not only added topographical features to the otherwise smooth bulk hydrogel but also provided binding regions for sequestering compounds with hydrophobic sites, such as proteins, as verified using an extrinsic hydrophobic fluorescent probe. In-situ and on-demand multi-functionalization was confirmed by performing consecutive SPAAC conjugations. Reactions proceeded rapidly (< 30 min) under physiological conditions (i.e., in culture medium at 37°C). In MSC-laden ALK hydrogels, cells remained viable and metabolically active throughout culture time. Cell spreading and extracellular fibronectin expression were detected at the microdomain regions only, which worked as topographical harbors for cell anchoring.

By taking advantage of the intrinsic hydrophobicity of cyclooctyne groups, which can be conjugated with azido-conjugated compounds via SPAAC, we successfully formulated hydrogels that present topographical cues to cells in a true 3D microenvironment, while also allowing dynamic, on-demand, (bio)functionalized in the presence of cells.

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keywords: amphiphile alginate, SPAAC, hydrophobic domains, topography

73296354639

ENGINEERING CELL-INSTRUCTIVE MICROENVIRONMENTS USING INJECTABLE, TOPOGRAPHICALLY-TEXTURED POLYMERIC MATRICES

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Injectable biomaterials have evolved from serving as simple structural fillers to acting as multi-functional systems capable of directing human mesenchymal stromal cell (hMSCs) response. However, the selection of appropriate design parameters for injectable polymeric microbeads for translational applications remains a challenge. We have demonstrated promising strategies to address this need by tailoring the architectural features of injectable microbeads to act as modulating moieties of attachment and osteogenic response in hMSCs [1, 2].

Topography offers a vital tool to be harnessed for guiding cell fate, since topographical features tend to be more robust than surface chemistry and can be modified in terms of size, shape and degradation rate. We have demonstrated that topographical patterning of polylactic acid microbeads offers cell-instructive 3D microenvironments to allow the modulation of hMSCs fate by eliciting the desired downstream response without adding exogenous bioactive supplements. Topographically-patterned microbeads of varying microscale features (acting as braille for cells) were produced by phase separation of a sacrificial component from polylactic acid during fabrication. We established that culturing hMSCs on dimpled microbeads recreates mechanical aspects of the endosteal niche and exhibited varying morphological, integrin-mediated adhesion and proliferation responses. Additionally, significantly increased expression of osteogenic markers in hMSCs cultured on dimpled microbeads relative to conventional smooth microbeads was observed in the absence of exogenous biochemical factors. The cells also exhibited significantly altered metabolic profiles on different microbeads designs and resulted in varying histological characteristics in vivo [1]. Surface-functionalised textured microbeads were used to investigate the relative importance of surface chemistry over topography on the formation of 3D hMSCs-microbeads aggregates for 3D culture applications [2].

Our work delivers new guiding principles for the design of 3D cell-material interfaces, and opens up new avenues for engineering tailored injectable materials for applications spanning regenerative therapies, disease models, cell culture and advanced cell delivery systems.

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keywords: Bone engineering; Mesenchymal Stromal Cells; Polymeric microparticles; Microbeads; Topography

S39
Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering
Room: S2
(30 Jun 2022, 11:00 - 12:30)

Conveners:
Beata Niemczyk-Soczynska; Paweł Sajkiewicz

73296353379

INJECTABLE AND PHOTOCURABLE AMPHIPHILIC HYBRID NETWORKS: SYNTHESIS APPROACH USING NON-TOXIC CATALYSTS

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Introduction:
Injectability is one of the most desirable features of biomaterials. The combination of injectability and photocuring can provide materials to be easily and safely delivered using minimally invasive procedures. This is especially important for tissue sealants which allow to exclude the use of sutures or staples or for patches supporting the weakened tissue. Therefore, the aim of presented work is to create new injectable amphiphilic polymer-polymer hybrid system, which can be UV-cured in situ in vivo to form flexible patches for soft tissue regeneration. Such systems, with sufficient adhesive properties can be used without the need of using sutures or tacks and by applying them via minimally invasive procedures to provide support for the tissue.

Materials and Methods:
The polymer-polymer hybrid networks were prepared from fatty acid-modified precursors (Pr) bearing methacrylic groups and from PEGylated fibrinogen. For the synthesis of telechelic fatty acid-based macromonomers, three catalytic systems were tested: bismuth tris(2-ethylhexanoate) (BiHex) and zinc (II) acetyloacetonate (ZnAc), both in 4 mol%, and organo-Mg-Ti catalyst (1 mol%). The obtained liquid monomers were characterized by NMR and GPC. The UV-curing has been performed at the wavelength λmax of 385 nm (LED source) turning the liquids into flexible solids with use of 2% w/w photoinitiator (Omnirad 819). The gel fraction has been determined by refluxing materials in DCM. Cytotoxicity tests were performed on extracts using L929 cell line. Cell viability was then assessed using light microscopy and the resazurin viability assay on samples UV-cured in air and in argon. The PEG-ylation of fibrinogen was performed in two steps. In the first step, fibrinogen was dissolved in 50mM PBS with 8M urea (protein concentration 7mg/ml) and TCEP HCl was added and then stirred for 2h. After that, PEG-DA 4kDa was added (145:1 PEG-DA: fibrinogen) and reaction was continued overnight. Obtained product was purified and characterized by NMR (Bruker DPX HD-400 MHz). The hybrid was prepared using telechelic macromonomers and PEG-ylated fibrinogen with photoinitiator followed by solvent evaporation. Photocuring was performed in air atmosphere with use of the same UV-light source.

Results and Discussion:
New catalytic systems allowed to obtain telechelic macromonomers at the shortest reaction times for bismuth (7h) and magnesium-titanium catalysts (9h). Rection yield was similar for all of the materials (65-57%). The cell viability study showed that the use of nontoxic catalysts resulted in high cell viability, regardless of gel fraction. PEGylation of fibrinogen has reached high yield of 88%. The use of co-solvents, here ethyl acetate and/or dichloromethane and/or dimethylsulfoxide allowed to prepare amphiphilic hybrids. Their characteristics and adhesive properties will be discussed during the lecture.

Conclusions:
Photocurable macromonomers were synthesized with new catalysts, being non-toxic as

revealed by high cell viability. The use of co-solvents allowed to prepare amphiplilic networks from hydrophobic macromonomer and hydrophilic PEGylated fibrinogen with the use of UV light. Obtained hybrid networks showed also high cell viability and elastomeric properties thus manifesting their suitability as patches for soft tissue engineering.

Acknowledgements:
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This is an invited talk to the symposium "Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering"

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keywords: Injectable biomaterials, elastomers, UV curing, amphiphilic polymer networks

94238107637

INJECTABLE THERMOSENSITIVE METHYLCELLULOSE/AGAROSE HYDROGEL AS SMART SCAFFOLD FOR TISSUE ENGINEERING APPLICATIONS

Beata Niemczyk-Soczynska (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Dorota Kolbuk (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Grzegorz Mikulowski (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland), Piotr Rogujski (Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland), Luiza Stanaszek (Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland), Pawel Sajkiewicz (Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland)

Introduction
The aim of this research was to design and obtain an injectable thermosensitive hydrogel consisting of methylcellulose (MC) and agarose which would serve as a smart scaffold for tissue engineering applications. The MC provides thermal sensitivity, while heated up to c.a. 37°C becoming a physically crosslinked hydrogel. While agarose enhances crosslinking of MC rate and increases its mechanical properties. To evaluate the usefulness of such an injectable thermosensitive hydrogel system from the perspective of tissue engineering applications, injectability studies and biological tests with the use of two cell lines were carried out.

Methodology
In these studies, the injectability of MC/agarose hydrogel was studied using a dynamometer system, in which the maximum force needed for making injections was measured and compared to the literature reports. Additionally, in vitro cellular studies were performed using fibroblasts and mesenchymal stem cells (MSCs). Cellular morphology was analyzed via scanning electron microscopy (SEM) and fluorescence microscopy (FM), while a cytotoxicity test was carried out on extracts using the Presto Blue assay.

Results
The injectability tests showed the maximum force needed for making the injection of MC/agarose hydrogel was less than 30 N which according to Kim et al. [1], is the maximum force required for injection by a human. Biological studies showed proper cell morphology in comparison to control (tissue culture plastic -TCP). Additionally, cytotoxicity tests confirmed the nontoxic character of studied hydrogel systems.

Conclusions
Injectability studies showed the investigated MC/agarose hydrogel systems might be readily injected by humans, proving their injectability. Investigated MC/agarose hydrogels provided a hospitable ECM-mimicking environment enhancing cell spreading, migration, and proliferation. These studies demonstrate the high potential of investigated materials for tissue engineering applications.

Acknowledgments: Financial support was received from Polish National Science Center (NCN), grant number 2018/29/N/ST8/00780.

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keywords: injectable, hydrogel, methylcellulose, agarose

62825411166

ENZYME-CONTROLLED, NUTRITIVE HYDROGEL FOR MESENCHYMAL STROMAL CELL SURVIVAL AND PARACRINE FUNCTIONS

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Introduction:
Human Mesenchymal stromal cells (hMSC) are appealing candidates for regenerative medicine applications. However, upon implantation, they encounter an ischemic microenvironment depleted of oxygen and nutrients responsible for their massive death post-transplantation, a major roadblock to successful clinical therapies. To date, various approaches have been proposed to address this issue, albeit with limited clinical success. We hereby propose a paradigm shift for enhancing hMSC survival by designing, developing, and testing an enzyme-controlled, nutritive hydrogel with an inbuilt glucose delivery system for the first time. This novel hydrogel is composed of fibrin, wheat starch (a glucose polymer), and amyloglucosidase (AMG), which hydrolyze glucose from starch.

Methodology:
In vitro: Glucose concentration at the core of hydrogels was determined using a custom-made glucose electrode biosensor. hMSC survival was assessed by cytometry after releasing cells from cell-loaded hydrogels exposed at 0.1% oxygen for up to day 14. The chemotactic potential of hMSCs towards hMSC and Human Umbilical Vein Endothelial Cells (HUVEC) was assessed by collecting conditioned Media (CM) from these hMSC-loaded hydrogels and evaluating migration in Boyden chambers. Moreover, chemotactic and angiogenic cytokines in CM were quantified using Luminex®.
In vivo: fluorescent-labelled AMG leakage from cell-free fibrin hydrogels was monitored using Xenogen live imaging until day 14 after ectopic implantation in nude mice. Luciferase-labelled hMSC survival within both fibrin/starch/AMG and fibrin hydrogels was assessed in an ectopic nude mice model by bioluminescence imaging until day 14. New blood vessel formation in the hydrogel vicinity was determined by µCT scanner, using a radiopaque agent Microfil® perfused within blood vessels at day 7 and 14.
Results:

In vitro fibrin/starch/AMG hydrogels released glucose at physiological concentrations and exhibited a 95 fold increase in hMSC survival compared to fibrin hydrogels after 14 days. CM collected from hMSC loaded fibrin/starch/AMG hydrogels showed (i) a 9- and a 4-fold increase in chemotactic potential towards hMSCs and HUVECs and (ii) a statistically significant rise in most but not all chemotactic and angiogenic cytokines compared to hMSC loaded fibrin hydrogels. In vivo glucose concentration within cell-free fibrin/starch/AMG hydrogels was within physiological ranges at days 7 and 14. Fluorescence monitoring revealed that AMG had completely disappeared within 7 days. hMSCs viability (measured by bioluminescent signal intensity compared to day 1) was 76.4% within fibrin/starch/AMG hydrogels and 22.1% within fibrin hydrogels at day 7. The hMSCs viability decreased drastically between days 7 and 14, corroborating the AMG time course. Last but not least, the formation of new blood vessels in the hydrogel vicinity exhibited a 4-fold increase when using fibrin/starch/AMG hydrogels compared to fibrin hydrogels at day 21.

Conclusion:
We hereby establish the proof of concept that a fibrin/starch/AMG hydrogel provides glucose to hMSCs and maintain their viability and angioinductive potential in vitro and in vivo. AMG sustained delivery is required to extend the survival time of the transplanted hMSCs.

Author 1 and 2 Contributed equally to this work and should be considerate as co-first

keywords: Mesenchymal stromal cells, regenerative medicine, nutritive hydrogel, viability and paracrine functions, survival

94238162648

ASSESSING EFFICACY OF REGENERATIVE THERAPIES FOR ISCHAEMIC STROKE - A NOVEL APPROACH FOR MORE MEANINGFUL OUTCOMES IN PRECLINICAL MODELS

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Introduction. Despite being the youngest branch of regenerative medicine, neural tissue engineering has rapidly developed, with numerous advances close to clinical translation. One of the most researched areas are tissue engineering and regenerative medicine approaches for brain repair after ischaemic stroke, with more than 70 preclinical studies testing therapeutic combinations of biomaterials, stem cells, and/or therapeutic proteins. Many of these studies have administered the regenerative therapies at the target site, in the stroke cavity resulted following the clearance of dead tissue in the chronic phase of stroke. This localised administration favours tissue repair by overcoming the blood brain barrier, which drastically limits drug accessibility into the brain. Most studies assess behavioural and histological outcomes. Here, we propose a more specific approach to assess the success of a combination therapy based on the use of an injectable synthetic peptide hydrogel and two multifunctional therapeutic proteins for inducing recovery following ischaemic stroke.

Methodology. We propose outcome evaluation at three main levels: behaviour functional (through sickness, wellbeing, and memory behavioural tests), functional vascular, and histological level. The behaviour functional level assesses brain function, representing the ultimate goal of regenerative therapies. In this regard, sickness, wellbeing, sensory-motor, and memory tests are performed. The functional vascular level assesses the cerebral blood flow in potentially regenerated areas through magnetic resonance imaging (MRI) arterial spin labelling (ASL). The histological level assesses markers of interest for the main repair processes which should be targeted by novel therapies: reduction of inflammation, immune-modulation, angiogenesis, and neurogenesis.

Results. Here, we have assessed the effect on functional recovery of a regenerative construct using wellbeing and sickness behaviour tests like the burrowing and nest building tests, and the sensorimotor neuroscore test. Functional vascular assessment through MRI ASL can discern cerebral blood flow differences after stroke, differences which minimise at 28 days post stroke. Histological assessment of brain inflammation was done through scoring of brain immune cells, Iba1+ (ionising calcium binding adaptor molecule 1) microglia, into discrete activation states. Relevant for tissue repair, measurement of glial scar thickness was made by GFAP+ (glial fibrillary acidic protein) integrated density quantification. Assessment of angiogenesis, the most targeted repair process in stroke, was done by measuring the ratio between endoglin, marker of new endothelial cells, and PECAM-1, an endothelial cell adhesion molecule. Finally, neurogenesis is assessed by integrated density of doublecortin positive cells infiltrated into the infarct and ratio of ipsilateral to contralateral doublecortin integrated density in the neurogenic niche-containing brain ventricles.

Conclusions. Our approach proposes the stroke outcome assessment in pre-clinical models at three different levels, behaviour functional, vascular functional, and histological functional.

These are instrumental in evaluating the success of regenerative therapies for brain recovery after stroke, before further progression into clinical practice.

keywords: stroke, pre-clinical models, hydrogel, biologics

31412743105

DESIGNING BIOINSPIRED MEDICAL ADHESIVES FROM MARINE BIOPOLYMERS AND TANNIC ACID

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Adhesive biomaterials have been studied by the scientific community in an attempt to surpass the current disadvantages of sutures and staples in surgery, such as the challenging implementation in some tissues and the risk of infection. The developed bioadhesives have been used as: i) glues for maintaining biological tissues together after laceration; ii) tissue sealants for preventing the leakage of body fluids, and; iii) hemostatic agents for helping generate the blood clot[1]. Despite the potential of this area, whose market size was valued at USD 1.8 billion in 2015 and is projected to grow 9.7% annually[1], existent adhesive materials approved by the Food and Drug Administration (FDA), still present several limitations. Some are not biocompatible or their degradation produces cytotoxic by-products, others lack bulk strength and bioactive properties, and some have proinflammatory potential[2].
Regarding wet-adhesion, mussel-inspired bioadhesives have gained attention, mimicking the mussel´s strong underwater adhesion, using catechol groups in the compound's structures[3]. Tannic acid (TA), a plant-derived polyphenol, is a safe and low-cost source of catechol/pyrogallol groups. It allows polymeric crosslinking through hydrogen and ionic bonding, or hydrophobic interactions, improving biomaterials adhesiveness and mechanical performance, while endowing it with anti-microbial, anti-inflammatory and antioxidant properties[4]. Hence, by combining laminarin (LAM-OH) or pullulan (PUL-OH), two natural origin polisaccharides with TA, bioinspired adhesive biomaterials for biomedical applications were produced.
A library of bioadhesives was fabricated by combining PUL-OH and LAM-OH with TA in several concentrations, having the best formulations been chosen by lap shear test performance. Then, LAM-OH and PUL-OH were functionalized with methacrylic groups, having the modification of the polymers backbone (LAM-MET and PUL-MET) been successfully confirmed by 1H-NMR spectroscopy and FTIR. In the previous best formulations, the natural polysaccharides were substituted by LAM-MET and PUL-MET, respectively, and the bioadhesives presented adhesion to wet porcine skin, contrary to some already commercialized cyanoacrylate adhesives. Rheological and biological properties were also evaluated. Therefore, the present bioadhesives show good perspectives for being implemented as soft tissue bioadhesives.

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keywords: Marine polysaccharides; Tannic acid; Bioinspired; Bioadhesives; Biomaterials

41883642006

DRUG-LOADED ALGINATE MICROSPHERES FOR BREAST CANCER TREATMENT

Delia Catalano (Politecnico di Milano, Milan, Italy), Sophia Dalfino (Politecnico di Milano, Milan, Italy), Matteo Pitton (Politecnico di Milano, Milan, Italy), Silvia Farè (National Consortium of Materials Science and Technology, Milan, Italy)

Introduction. Breast cancer is considered nowadays the most common cause of death for female population. Since traditional treatments (e.g., chemotherapy, radiotherapy, surgery) showed several drawbacks [1], Drug Delivery Systems (DDS) recently gained interest due to the possibility to release therapeutic agents locally and controllably in targeted sites. Herein, we aimed to develop stable drug-loaded alginate microspheres (MS) encapsulating a natural anti-tumoral drug, curcumin, to be released in the tumor site [2]. Unloaded and drug-loaded MS were investigated by a morphological, chemo-physical, and biological characterization.

Materials and Methods. Sodium alginate (3% w/V) was dissolved in distilled water and alginate MS were obtained by extrusion dripping, using a coaxial needle (dint = 26G, dext = 20G), connected to a compressor which allowed a laminar air flow (P = 0.5 bar). Alginate droplets were chemically crosslinked in a CaCl2 (450, 900 mM) bath, and MS were then collected. In vitro stability tests were carried out on curcumin-loaded (0.3% w/V) and unloaded MS formulations, in neutral (pH = 7.4) and acidic (pH = 5.3) environment. Then, curcumin-loaded alginate MS were investigated in terms of encapsulation efficiency (EE%) and drug release. Lastly, in vitro biological tests were performed to investigate the effect of curcumin-loaded alginate MS on MCF-7 tumoral cells, previously 2D-seeded.

Results and Discussion. Extrusion parameters were selected through MS morphological analysis (threshold: Ø < 1.5 mm, circularity > 0.6) [3] [4]: A3-C450-0.5, A3-C900-0.5. In vitro tests stability exhibited no significant differences ($p > 0.05$) between alginate MS formulations in the same environment. This finding suggested that alginate MS reached the maximum swelling when crosslinked in 450 mM CaCl2. Differences ($p < 0.05$) were noticed comparing swelling kinetic in the two environments: degradation rate appeared faster (130 h) at pH 7.4 than at pH 5.3 (1008 h). EE% resulted higher ($p < 0.05$) for A3-C450-0.5-Cur than for A3-C900-0.5-Cur. As regards, alginate instantaneous crosslinking allowed for a higher drug release from MS, diminishing EE% [5]. Furthermore, drug release tests did not show significant differences ($p > 0.05$) between two CaCl2 concentration. Otherwise, the environment allowed for a lower drug release in acidic environment for both the formulations. In vitro biological tests showed that MCF-7 tumoral cells exhibited an increasing metabolic activity when cultured in contact with drug-unloaded alginate MS, as expected. Cell metabolism decreased when MCF-7 cells were cultured in presence of curcumin-loaded alginate MS.

Conclusions. Optimized alginate MS were tested as DDS in our work to develop a stable and controllable anti-tumoral therapy for breast cancer treatment. As regards, in vitro biological tests showed DDS efficiency in terms of drug release, which resulted time prolonged and specific. As regards, MCF-7 tumoral cells cultured on TCPS decreased their cell metabolism once in presence with curcumin-loaded MS.

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keywords: drug delivery, breast cancer, alginate microspheres, extrusion dripping

94238135166

ADVANCED STEM CELL THERAPY FOR NEURODEGENERATIVE DISEASES

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Introduction
Neurodegenerative diseases (NDs) are a group of chronic disorders (e.g. Alzheimer's disease, Parkinson's disease and multiple sclerosis -MS) characterized by progressive neurological dysfunction. Despite different neuronal populations can be affected, NDs share major clinical manifestations, namely motor impairment, cognitive disability and/or dementia. Effective treatments do not exist, but stem cell therapies emerged as treatment modalities with potential to cure NDs. However, despite high initial expectations, their clinical use is still limited. To overcome their crucial limitations, such as poor cell survival and low penetration into the central nervous system (CNS), we designed a hydrogel to deliver bone marrow mesenchymal stem cells (BMSCs) intrathecally or intracerebroventricularly.

Methodology
The hydrogel physically crosslinked with liposomes was based on biomolecules (phospholipids and hyaluronic acid-HA) naturally present in the CNS. The characterization comprised the determination of size, polydispersity index (PDI), surface charge and temperature transition of large unilamellar liposomes (LUVs) and their distribution in the HA matrix as well as the assessment of the gel's thermal and rheological behaviors. Hydrogel cytocompatibility was assessed using BMSCs isolated from healthy rats. To determine biocompatibility and efficacy two rat strains were used, namely Wistar Han rats and Lewis rats, respectively. This choice was based on their ideal use for testing the formulations' safety and therapeutic efficacy in experimental autoimmune encephalomyelitis (EAE). The assessment of the hydrogel's in vivo compatibility was performed through its direct injection into the rat's ventricular space. Moreover, a fluorescent-labeled hydrogel was used to investigate its brain distribution. To determine the efficacy of the developed formulation, containing a significantly lower number of cells than previously reported, the daily body weight, clinical score, and neuropathology levels were assessed in EAE rat models.

Results
LUVs presented a homogeneous (PDI=0.088+0.022) size of 115.7+3.5 nm and a significant negative surface charge (-33.4+3.7 mV). Hydrogels with LUVs displayed a rougher surface than the glycosaminoglycan hydrogel. The shift to a lower value of the temperature of the endothermic peak of HA also confirmed the presence of liposomes. Moreover, liposomes increased the elastic and viscous moduli of the HA matrix as well as the viscosity of the formulation. The encapsulation of BMSCs in the 3D matrix demonstrated they were able to adhere to, survive and proliferate within the hydrogels to a higher extent than in 2D cultures. In vivo studies confirmed hydrogel safety. Moreover, the hydrogel diffused into the corpus callosum, which is ideal for NDs treatment, as the damage of this white matter structure is responsible for important neuronal deficits. The BMSCs-laden hydrogel significantly decreased the maximum mean clinical score and average mean clinical score when compared with the

control group of EAE and eliminated the relapse.

Conclusions
The developed formulation was more efficacious in reducing disease severity and maximum clinical score in EAE rats than cells suspensions, demonstrating the added value of cell incorporation in the hydrogel. Therefore, the engineering of stem cells therapies using this natural carrier can result in efficacious treatments for MS and related debilitating conditions.

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keywords: Neurodegenerative diseases, multiple sclerosis, bone marrow mesenchymal stem cells, hydrogel, CNS delivery

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HA AND PRP COMBINATIONS AS “OFF THE SHELF“ DEVICE FOR CLINICAL APPLICATIONS

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INTRODUCTION
The regenerative effect of Platelet Rich Plasma on skin and other tissue lesions is well known. If, on the one hand, research aims to optimize PRP standardized protocols, on the other hand, it aims to identify substrates as vehicles for the platelet content release to the lesion site. For the latter purpose, hyaluronic acid (HA) is proposed thanks to its viscoelastic and biological properties and biocompatibility. The aim of this study was the set-up and characterization of an “off the shelf” freeze-dried and injectable device based on HA that entraps PRP in a stable matrix sustaining the platelet growth factor release.

METHODOLOGY
High MW HA (1400 KDa – HA-HMW) and Low MW HA (56, 90, 200 KDa – HA-LMW) were used in combination with PRP at the platelet concentration of 2.5x106 plts/ul in the ratio 1:1. The Rheology of selected HAs with defined hydrodynamic parameters was analyzed at the concentration used for PRP mixing and lyophilization. After regeneration, the resulting lyophilized mixtures HA/PRP were tested for in vitro cell proliferation and scratch assays on human primary fibroblasts. The biological activity of freeze-dried HA-LMW/PRP formulations were also tested during storage at different temperatures (25°C, 4°C and -20°C) up to 6 months.

RESULTS
In a first set of experiments, HA-HMW/PRP was evaluated for its biological activity, showing that the freeze-dried and regenerated HA-PRP combination supported human dermal fibroblast proliferation in a comparable way to PRP alone. Although the biological properties of the HA-HMW/PRP were maintained, the formulation needed almost half an hour for full regeneration and quite a strong pressure to be extruded by a 21-gauge needle.
To overcome these limitations, hyaluronans of low molecular weight were selected after a specific hydrodynamic (SEC-TDA) analysis, namely HA 56 KDa, HA 90 KDa, HA 200 KDa. All formulations obtained by the combination of these HA with PRP induced cell proliferation. For a clinical application of an “off the shelf” lyophilized product it is mandatory to preserve the PRP activity along time. We already reported that the long-term storage of the freeze-dried PRP was associated to a progressive biological activity loss. In this work, the HA/PRP formulation were tested to evaluate the possible stabilization by HA at different temperatures and length of storage.
All formulations induced cell proliferation comparable to PRP alone at the different tested temperatures, but, interestingly, the 56 KDa HA/PRP formulation, after 6 months of storage

at 25°C, showed significant preservation of the proliferation activity compared to PRP alone, suggesting a protective effect of HA versus the PRP bioactive factors. In addition, some of the low molecular weight HA-PRP formulation showed, at the same storage condition, a superior healing rate in a scratch assay followed by time lapse video microscopy.

CONCLUSIONS
In conclusion, we developed a lyophilized HA-based/PRP device, that may improve bioadhesive properties of the sole PRP also improving on site delivering (e.g. wound treatment). These formulations proved to release platelet factors preserving their biological activity over time. HA/PRP allows the development of promising products, for topical and intra-articular applications.

keywords: Hyaluronic acid, Platelet Rich Plasma, freeze-dried, injectable device

S40
Injectable scaffolds in tissue engineering
Room: S2
(30 Jun 2022, 15:30 - 17:00)

Conveners:
Beata Niemczyk-Soczynska, Irene Lara-Saez

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SCAFFOLDS FOR THE DELIVERY OF GENE THERAPEUTICS FOR ENHANCED TISSUE REPAIR

Fergal O'Brien (RCSI, Dublin, Ireland)

The COVID-19 pandemic has shown how revolutionary treatments based on gene therapeutics has helped overcome a once-in-a-century pandemic and has given new momentum to gene therapy research for a myriad of applications. The field of regenerative medicine is well placed to be a beneficiary whereby, for example, gene therapy might be a valuable tool to avoid the limitations of local delivery of growth factors. While non-viral vectors are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated gene therapy approach for regenerative applications. These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also can be engineered to direct autologous host cells to take up specific genes and then produce therapeutic proteins in a sustained but eventually transient fashion. Similarly, we have demonstrated how scaffold-mediated delivery of siRNAs and miRNAs can be used to silence specific genes associated with reduced repair or pathological states. This presentation will provide an overview of ongoing research in our lab in this area with a particular focus on gene-activated biomaterials for promoting bone, cartilage, nerve and wound repair. Focus will also be placed on advances we are making in using 3D printing of gene activated bioinks to produce next generation medical devices for tissue repair.

keywords: scaffolds, gene delivery, nanomedicine

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INJECTABLE HYDROGELS FOR JOINT REGENERATION

Catherine Le Visage (Inserm Regenerative Medicine and Skeleton, Nantes, France)

The development of hydrogels for regenerative medicine has progressed to the point where they are now considered one of the best options for successfully regenerating injured tissues. Their structural similarities to the extracellular matrix (ECM) and their versatility make them excellent candidates to mimic a native environment. Indeed, they are easily chemically modified and can be tuned to exhibit adequate degradation profile and mechanical integrity, as well as to incorporate growth factors or cytokines, making them suitable microenvironments to guide cell infiltration, proliferation, migration and differentiation, as well as innovative delivery systems of cells, extracellular vesicles or nucleic acids. Injectable hydrogels are the most extensively studied as they offer unmatched advantages compared to other biomaterials. As fluid materials, they have the ability to set in situ by physical or chemical crosslinking to form 3D microenvironments, thus simplifying their injection during minimally invasive surgery. Moreover, the development of dynamic chemistries now allows the use of hydrogels as nearly-physiological matrices to recapitulate the dynamic interactions of native environments.
In this talk, we will first provide an overview of the polymers, chemistries and fabrication techniques that are used to develop injectable hydrogels. We will highlight promising strategies that are used for tissue regeneration, notably in the field of joint diseases. We will then look at cell microencapsulation approaches with natural polymers (hyaluronic acid, alginate). Recent advances in droplet-based microfluidics and micromolding technologies will be discussed. We will also highlight the requirements in terms of diffusion and size properties, outline the 3D microenvironments we have recently developed in our lab using soft lithography technique, and discuss their relevance in the context of osteoarthritis and intervertebral disc treatment.

keywords: Hydrogels, skeletal, joint, intervertebral disc, 3D microenvironment

62825420404

LOW-INTENSITY PULSED ULTRASOUND DIRECT CHONDROGENIC DIFFERENTIATION OF ADIPOSE-STROMAL CELLS IN 3D PIEZOELECTRIC HYDROGELS

Cristina Manferdini (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Elena Gabusi (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Paolo Dolzani (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Yasmin Saleh (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Diego Trucco (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Marta Columbaro (IRCCS Istituto Ortopedico Rizzoli, Direzione Scientifica, Bologna, Italy), Lorenzo Vannozzi (The BioRobotics Institute, Scuola Superiore Sant'Anna; Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Pisa, Italy), Andrea Cafarelli (The BioRobotics Institute, Scuola Superiore Sant'Anna; Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Pisa, Italy), Enrico Lenzi (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy), Leonardo Ricotti (The BioRobotics Institute, Scuola Superiore Sant'Anna; Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Pisa, Italy), Gina Lisignoli (IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy)

Introduction: A major challenge in cartilage tissue engineering (TE) is to develop scaffolds capable of providing an instructive biomimetic environment to effectively drive mesenchymal stromal cells (MSCs) differentiation [1]. Hydrogels have emerged as promising biomaterials for this purpose, due to their biocompatibility and ability to mimic the tissue extracellular matrix [2]. Recently, graphene oxide (GO) emerged as a promising nanomaterial for cartilage TE due to chondroinductive properties when embedded into polymeric formulations [3]. It has been also shown that piezoelectric nanomaterials, like barium titanate (BaTiO3) nanoparticles, can be exploited as nanoscale transducers capable of inducing cell growth/differentiation [4]. Ultrasound waves are an interesting tool to facilitate chondrogenesis. In particular, it has been demonstrated that low-intensity pulsed ultrasound (LIPUS) regulates the differentiation of adipose mesenchymal stromal cells (ASCs) [5].
The aim of this study was to investigate whether dose-controlled LIPUS is able to direct chondrogenic differentiation of ASCs embedded in a 3D piezoelectric hydrogel.

Methodology: Human adipose mesenchymal stromal cells at 2*106 cells/mL were embedded in 3D VitroGel RGD® hydrogel with or without nanoparticles (GO, 25µg/ml, BaTiO, 50µg/ml) and exposed to LIPUS stimulation (frequency: 1 MHz, intensity: 250 mW/cm2, duty cycle: 20% , pulse repetition frequency: 1 kHz, stimulation time: 5 min) every 2 days, until day 10 of culture. Hydrogels were cultured and chondrogenic differentiated for 2,10 and 28 days. At each time point cell viability (Live&Dead), cytotoxicity (LDH), gene expression of collagen type 2 (COL2), aggrecan (ACAN), SOX9, and collagen type 1 (COL1), electron microscopy, histology and immunohistochemistry (COL2, aggrecan, SOX9, and COL1) were evaluated.

Results: In both 3D hydrogels we evidenced that LIPUS treatment did not affect negatively the viability of the embedded cells. LIPUS boosted the chondrogenic differentiation of ASCs laden in 3D piezoelectric hydrogel: the chondrogenic genes and proteins markers (COL2, aggrecan and SOX9) were increased while the fibrotic marker COL1was decreased compared to control samples (non piezoelectric hydrogels and piezoelectric hydrogels not stimulated with LIPUS).

Conclusions: These results suggest that the combination of LIPUS and piezoelectric hydrogels push the differentiation of ASCs encapsulated in a 3D hydrogel and represent a promising tool in the field of cartilage TE.

keywords: Low-intensity pulsed ultrasound (LIPUS), Adipose mesenchymal stromal cells (ASCs), Hydrogels Chondrogenic differentiation

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CHARACTERIZATION AND MOLECULAR IMAGING OF A BIOHYBRID TISSUE ENGINEERED VASCULAR GRAFT

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Introduction:
The high number of vascular diseases demand for vascular grafts in various clinical application. Nonetheless, a big hurdle for the use of autologous tissue-engineered vascular grafts (TEVG) is represented by the usually long manufacturing time.Therefore, we here present a biohybrid vascular graft and a bioreactor that can provide distinct conditions such as flow, pressure, and temperature allowing the in vitro bioreactor conditioning of TEVG within four days. For in situ monitoring, we established reliable non-invasive imaging methods to monitor the degradation of the synthetic structural elements, ECM production, and signs of inflammation by molecular magnetic resonance imaging and ultrasound.

Methods:
A polyvinylidene fluoride (PVDF) tubular textile mesh was used as a permanent scaffold and coated with biodegradable superparamagnetic iron oxide nanoparticles (SPIONs) labeled PLGA fibers. TEVGs were prepared by a molding process which consists of the scaffold, fibrin gel, and arterial smooth muscle cells (SMCs). After molding an endothelialization process and bioreactor conditioning mimicking physiological blood flow and pressure values followed. Burst strength and suture retention strength of TEVG were measured and compared before and after bioreactor conditioning. The ECM production was studied in TEVG after 14 days of maturation using elastin- and collagen type I-targeted MR molecular gadolinium-based probes and immunohistology. The αvβ3 integrin expression as a marker of inflamed endothelium was assessed by molecular targeted US using RGD-poly(butyl cyanoacrylate) microbubbles and compared to RAD-control microbubbles1.

Results:
After four days of conditioning in a close loop bioreactor, 617±85 mm Hg of burst pressure and 2.24±0.3 N of suture retention strength were achieved. The bioreactor provided a suitable environment to the TEVG in which the cells could proliferate and produce extracellular matrix. The immunohistological findings proved the development of smooth muscle actin, Collagen I, Collagen IV, and continuous endothelial linings within the TEVG's lumen. The presence of collagen was further identified by MRIusing a targeted contrast agent. The expression of integrin in TEVG was identified by selective binding of RGD microbubbles only after mimicking an inflammatory state.

Conclusion:
We introduce a biohybrid TEVG with a coated scaffold for longitudinal monitoring by non-

invasive molecular imaging methods. After 4 days of bioreactor cultivation, this graft provides sufficient stability for implantation and the possibility of longitudinal monitoring in situ.

1. Rama, E. et al., Adv. Sci. in publication (2022).

keywords: Tisuue engineering, Vascular graft, Biohybrid Implant, Multimodal Imaging, Bioreactor

62825435106

DESIGNING INJECTABLE PEPTIDE-BASED HYDROGELS FOR TERM APPLICATIONS

Alberto Saiani (University of Manchester, Manchester, United Kingdom)

Introduction
The use of non-covalent self-assembly to construct materials has become a prominent strategy in biomaterial science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from cell culture and tissue engineering to in-vivo cell and drug delivery.[1] A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides. The beta-sheet motif is of particular interest as short peptides can be designed to form beta-sheet rich fibres that entangle and consequently form very stable shear-thinning (injectable) hydrogels. The intrinsic biocompatibility and low immunogenicity of these materials makes them ideal for TERM applications. [2-8]

Methodology
We explored the unique shear thinning properties (injectability) of a family of short beta-sheet forming peptides (8-10 amino acids long). Through in-depth structural characterisation (AFM, TEM, SAXS, FTIR) and detailed rheological studies (shear rheometry and SIPLI) and modelling of dynamic behaviour (Standard mechanical models) we were able to develop a fundamental understanding of how design affects injectability. This understanding was then used to develop injectable system for the delivery of cell for a range of TERM applications.

Results & Discussion
Due to the self- assembled nature and dynamic properties of this family of peptides we were able to design readily injectable systems. We showed how the beta-sheet fibre edges properties (hydrophilic vs hydrophobic) could be modified by adding lysine end-residues. This allowed to design highly dynamic systems that were able to "liquify" (shear-thin) upon application of a large strain (e.g.: pressure) and then recover instantaneously their gel-like properties upon removal of the strain. [3] We showed that these hydrogels allowed the successful delivery of cells through injections using very small needle gauges.
In recent work performed in the context of nucleus pulposus repair and heart regeneration using a rat model we showed the potential of these systems as injectable functional materials for TERM applications. [4-6] In addition, we also used 3D-briorinting approaches to show that these shear-thinning materials are ideal bioinks for the 3D printing of cells. [7-8]

Conclusion
The intrinsic biocompatibility and non-immunogenic nature of these system combined with their unique shear-thinning properties allowed us to develop a family or injectable system for TERM applications. We demonstrate how design rule can be manipulated to tailor the properties of these materials to the application intended.

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keywords: Scaffold, Injectable, Hydrogel, Peptide, TERM

73296368139

LIVER-SPECIFIC LIGAND-CONJUGATED MICROPARTICLES FOR TARGETED ISLET TRANSPLANTATION

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INTRODUCTION
Islet transplantation is a recognised treatment for type 1 diabetes: islets are transplanted into the liver and the procedure can stabilise blood glucose levels. A major limitation for successful clinical islet transplantation is the significant loss of islets post-transplantation due to an immune mediated inflammatory reaction. Etanercept, a TNF blocker that binds specifically to TNF-α, is currently provided as a systemic injection to patients on the first, third and fifth day post-transplantation. Whilst administration of etanercept has improved efficacy of clinical islet transplant, it is important to achieve targeted and controlled delivery to support islet engraftment locally. Encapsulation of etanercept within microparticles (MPs) may provide a suitable route for delivery. However, MPs are typically fabricated with commercially available polymers that are non-specific to the liver resulting in inefficient delivery.
Asialoglycoprotein receptor (ASGPR) exhibits high affinity as a galactose receptor and is the only liver-specific receptor identified on hepatocytes thus far. Herein, we demonstrate targeted delivery to the liver by synthesis of a novel poly(lactic-co-glycolic acid) (PLGA) polymer by covalent conjugation with galactose moieties. MPs fabricated from this polymer demonstrated sustained and controlled release of etanercept that reduced the inflammatory response suitable to promote islet survival.

EXPERIMENTAL METHODS
PLGA 85:15 (55 kDa) was functionalised with amino-functionalised lactobionic acid through amide bond formation in dimethyl sulfoxide for 16 h. One gram of galactosylated PLGA (Gal-PLGA) MPs encapsulated with 10 mg etanercept was fabricated using standard water-in-oil-in-water double emulsion method. In vitro functional drug release was measured using ELISA assay from aliquots of MPs suspended in PBS and gently rocked at 37 ºC for up to 7 days. Immunomodulatory response of MPs was performed using THP-1 differentiated macrophages. MPs were assessed in vivo following delivery via the portal vein.

RESULTS AND DISCUSSION
Gal-PLGA was synthesised to provide specific binding site to hepatocytes; MPs fabricated from Gal-PLGA provided an enhanced MPs retention (>85%, compared to 40% with conventional PLGA) in vivo. MPs fabricated with a mean size of 13 µm resulted in less hepatic necrosis when compared to larger particles. MPs exhibited a mean controlled daily release of 0.3 µg of protein per 1 mg of MPs between 2 and 7 days of in vitro release. Immunomodulatory response of MPs performed using macrophages suggest functional release of etanercept in the in vitro setting binds with TNF-α and potentially reduces inflammation observed by down-regulated pro-

inflammatory genes (CXCL2, IL-1β) as well as reduced release of soluble inflammatory cytokines (TNF-α, IL-6). Preliminary investigation in vivo has shown no adverse safety concerns in mice.

CONCLUSIONS
We demonstrated a novel synthesis route for galactosylation of commercial PLGA and the subsequent fabrication of Gal-PLGA MPs as a novel protein carrier for targeted liver delivery with controlled functional release kinetics of etanercept. Macrophages have shown reduced inflammation co-culture with etanercept MPs in vitro. Preliminary in vivo study has displayed no adverse safety concerns post-injection.

ACKNOWLEDGMENTS
This project is funded by the UK Regenerative Medicine Platform 2 (UKRMP2) [MR/R015651/1].

keywords: microparticles, islet, liver, diabetes, etanercept

20941881909

AN INJECTABLE HYDROGEL FROM A HYDROPHOBICALLY MODIFIED COLLAGEN FOR THE ENCAPSULATION AND DELIVERY OF FETAL CARDIAC MSCS

Mahsa Jamadi Khiabani (Macromolecular Chemistry, Department of Chemistry-Ångström, Uppsala University, Uppsala, Sweden), Ayan Samanta (Macromolecular Chemistry, Department of Chemistry-Ångström, Uppsala University, Uppsala, Sweden)

Statement of Purpose: The development of a hydrogel that could be injected and cured in vivo has gained increasing attention. Collagen has been widely investigated as a thermogel in which there are a lot of ionic interaction, hydrophobic interaction, and hydrogen bonding; however, it must be chemically crosslinked also. Diels-Alder cycloaddition occur under mild conditions without the need for any catalyst, toxic solvent or external activation like UV-irradiation which makes it a pragmatic choice for biomedical application and in particular for developing in situ crosslinked injectable gels. Hence, taking into account the kinetic characteristics of the Diels–Alder reaction, hydrophobic diene-terminated collagen was synthesized and then injectability experiments were performed on fabricated gels to investigate its potential applications in minimally invasive surgery.
Methods: Diene-terminated collagen was prepared employing the nucleophilicity of the ε-amino group of the lysine and arginine side chain. Hydrogels were then fabricated by mixing modified collagen stock solution with PEG maleimide in which the final concentration of modified collagen in the gel was considered to be 2% (w/v) To evaluate the injectability/shear-thinning properties of the hydrogels, viscosity under continuous flow was measured with increasing the shear rate (from 0.01 to 100 s^{-1}), using 20 mm stainless steel parallel plate geometry on hydrogels extruded directly on the rheometer plate from a syringe. Shear-thinning experiments were performed immediately, 4h and 48h after mixing all the hydrogel components. Furthermore, cardiac fetal stromal cells (CFSCs) were encapsulated in the gel network and cytocompatibility of the gels investigated with live/dead viability kit.
Results: Based on our experiments, all modified collagen gels were found to be extrudable (extrusion from a syringe) and injectable (extrusion through a 27G needle), whereas with increasing the shear rate, viscosity decreased. It should also be noted that linkage between diene and dienophile in Diels-alder reaction have dynamic nature. By increasing the temperature, the reaction rate of retro Diels-alder reaction increase, shifting the equilibrium towards the breaking of the reversible bonds. Additionally, instead of thermal energy, mechanical energy also makes the reversible polymer network becomes more dynamic, leading to retro Diels-alder reaction of cycloadducts into polymer chains. Our hypothesis is that injecting with needle act as a force-actuator, resulting in a mechanochemical coupling. From this point onward, the Diels-alder adduct should be coupled to the mechanical force which eventually trigger the retro Diels-alder reaction to release diene-terminated collagen and PEG-maleimide.
Conclusion: Given details mentioned above, the slow crosslinking of Diels-alder reaction and force-induced retro Diels-alder allows modified collagen gels to be injectable at least up to 48 hours post mixing of the gel components when stored at room temperature. Hence, this property is highly beneficial for potential clinical applications in terms of handling and administration given than the hydrogel could then be prepared prior to the surgery and then brought into the surgical room. Additionally, cell viability was higher than 80% for fabricated hydrogels for up to at least 7 days after cell encapsulation, suggesting that the engineered modified collagen gels had no cytotoxicity with CFSCs.

keywords: Collagen gel, Diels-alder reaction, Injectable gel

S41
Mesenchymal Stem / Stromal Cells – from basic research through clinical studies to registered products
Room: S3 A
(29 Jun 2022, 15:30 - 17:00)

Conveners:
Marcin Majka; Ewa Zuba-Surma;
Maria Rita Citeroni

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SURVIVING MESENCHYMAL STEM/STROMAL CELLS UPON INTRA-ARTICULAR DELIVERY IN AN OSTEOARTHRITIC JOINT EXPRESS A NEW CHONDROPROGENITOR GENE BMP/RETINOIC ACID-INDUCIBLE NEURAL-SPECIFIC PROTEIN 3 (BRINP3)

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Mesenchymal stem/stromal cells (MSCs) are considered a disease-modifying treatment for osteoarthritis (OA). However, the precise molecular mechanisms of actions under which MSCs exert their therapeutic effect have not yet been identified in OA. Since MSCs actively interact with their environment, most likely the inflammatory OA milieu will stimulate their response. To identify these mechanisms, we retrieved GFP+ bone marrow-derived MSCs after intra-articular (IA) delivery in a murine collagenase induced osteoarthritis (CIOA) model. The transcriptome of retrieved cells and control in vitro licensed GFP+ BM-MSCs were analyzed to identify the predicted secretome and potential novel therapeutic factors activated by the OA microenvironment.

CIOA was induced in C57BL/6 mice (n=8) and 2x105 mouse syngeneic GFP+ bone marrow derived MSCs (BM-MSCs) were IA injected at D14 and D56 representing early acute and late OA. For cell retrieval, knee joints were digested and isolated by FACS-coupled for RNA sequencing. Samples were compared to GFP+ BM-MSCs retrieved from SHAM joints, where the knee joints were injected with saline solution. BM-MSCs licensed in vitro with a single dose of interleukin 6 (IL-6) and a combination of IL-6, monocyte chemoattractant protein-1 (MCP-1) and interferon gamma (IFN- γ) were also analysed. After 72 hours, cells were processed for RNA sequencing. Validation of BMP/retinoic acid-inducible neural-specific protein 3 (BRINP3) as a new MSC marker was performed using indirect immunofluorescence staining in healthy and OA murine cartilage, mouse embryonic limb and in vitro chondrogenic differentiation in human MSCs and articular progenitor cells (ACPs).

BRINP3 was identified as a common element between the four groups and as a novel protein associated with MSC modulation. BRINP3 protein expression was validated, identifying positive signal in meniscal cells in healthy murine cartilage, and was also detected on the meniscal and articular cartilage surfaces in mouse models of OA and expressed in joint-forming locations and in the periosteal sleeve in the developing mouse limb. We further investigated BRINP3

expression during in vitro chondrogenic differentiation of MSCs and ACPs. Positive expression was identified in the cytoplasm of MSCs and at later stages of chondrogenic differentiation external to the cells suggesting active secretion. However, ACP signal was confined solely in the cell cytoplasm throughout all stages of cell differentiation.

We generated a database of predicted secreted genes that can be a valuable resource for identification of small molecules with potential therapeutic efficacy for OA treatment. Furthermore, the data provided insights of the therapeutic mechanisms of action of MSCs in the context of OA. Among secreted genes, we identified for the first time BRINP3 as a new protein expressed during embryonic limb development, in vitro chondrogenic differentiation and on the meniscal and articular cartilage surface in vivo where chondroprogenitor cells are located. The data also highlights a mechanism of action of with surviving MSCs taking on a chondroprotective role and future studies are needed to validate the potential of BRINP3 as a local treatment of OA.

keywords: mesenchymal stem cells, osteoarthritis, chondroprogenitor cells, cell retrieval, murine model.

20941845455

EFFECT OF DIFFERENT LIGHT WAVELENGTHS ON ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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Introduction: Treatment of cells with electromagnetic irradiation (light) can affect their proliferation and differentiation ability. Exposure of cells to light sources with different wavelengths (wavelengths around 415 nm/blue, 540 nm/red, and 810 nm/infrared are most common) appears to have different effects depending on the wavelength, energy intensity and the duration of exposure. Cytochrome c oxidase is believed to be one of the main photon acceptors. In this study, we have developed an experimental setup suitable for irradiating cells (with adipose tissue-derived mesenchymal stem/stromal cells/adMSC) with light of different wavelengths. With this setup, we are investigating the effect of light exposure on the regenerative capacity of adMSC.

Methodology: Since the cell culture media and growth surfaces have specific absorption properties, we measured absorption spectra of different media (with and without fetal calf serum/FCS, with and without phenol red) with different plate formats (clear bottom, with or without lid) and different volumes (50 µl, 100 µl, and 150 µl) using a microplate reader. Using the cell culture medium optimized for light treatment, the adMSC suspension was irradiated with the different wavelengths (blue (430 nm), red (660 nm) and IR (810 nm)) and different exposure times (5, 10 and 15 min). Subsequently, the phenotype, viability, cell number, cell cycle, mitochondrial membrane potential and differentiation ability of the cells were determined.

Results: Since the measurement with black plate with a flat transparent bottom without lid, showed little light scattering, these plates were used for the further analyses. The analysis of absorption spectrum of cell culture media showed that phenol red absorbs light with wavelengths below 600 nm. The media additive FCS showed similar absorption properties. The studies of different filling volumes showed that the absorption capacity increased in direct proportion with increasing filling volume. After optimizing the experimental setup, it was possible to carry out the first experiments on the irradiation of adMSC. Depending on the light source used, different effects occurred. These ranged from changes in cell morphology to reductions in cell number and metabolic activity. We also showed that the effect of light exposure on adMSC also depended on duration and energy input of the light. Further analyses to understand the energy and wavelength-dependent effect on cellular properties are still pending.

Conclusion: Since different materials and compounds have different absorption effects, the experimental setup needs to be adapted to the irradiation conditions. The composition of the media has a direct influence on the light absorption by adMSC, so it has to be adjusted accordingly. Preliminary experiments confirm that the viability and proliferation capacity of adMSC changes depending on the light wavelength and the energy input. With this background

knowledge, we can now examine the study of light effects on differentiation and migration ability. These examinations could help to transfer the light treatment to clinical application.

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[1] Wang, Yuguang et al, Scientific reports vol. 7,1 7781. 10 Aug. 2017
[2] Chen, Hongli et al, Lasers in medical science vol. 34,4 (2019)

keywords: adMSC, light wavelengths, absorption, cell differentiation

41883635884

MULTIPLE WHARTON JELLY MESENCHYMAL STEM CELLS-DERIVED HE-ATMP TRANSPLANTATIONS OVERCOMES DRUG-RESISTANT EPILEPSY IN CHILDREN

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Introduction
The wide spectrum of brain injuries experienced in neonates and preterm newborns and the potential plasticity of the CNS prompts us to seek solutions in the field of neuroregeneration in this group of patients and to prevent the worst effects of prematurity and perinatal problems. Drug-resistant epilepsy remains one of the biggest problems of prematurity and its consequences. There is a need among patients suffering from drug-resistant epilepsy (DRE) for more efficient and less toxic treatments than long time pharmacotherapy.

Method
The purpose of this study was to evaluate the safety and potential efficacy of multiple administrations of HE-ATMP comprised of 3x107 WJMSCs. A study group was composed of six patients, qualified for the treatment with a diagnosis of chronic hypoxic – ischemic encephalopathy and inflammation (including sepsis, and systemic inflammatory reaction) with diagnosis of DRE. All the patients underwent repeated rounds of HE-ATMP administration to the CSF via LP.

Results
There were no adverse events, and the therapy was safe and feasible over 2 years of follow-up. The therapy resulted in neurological and cognitive improvement in all patients, including a reduction in the number of epileptic seizures (from 40 per day to 2-5 per week) and an absence of status epilepticus episodes (from 4 per week to 0 per week). The number of discharges on the EEG evaluation was decreased, and cognitive improvement was noted with respect to reactions to light and sound, emotions, and motor function.
Conclusion

After two years of follow-up examination, we demonstrated the safety and beneficial effects of WJMSCSs transplantation, including neurological improvements and reduction of functional neurodeficits. We are aware that the samples size of this study is relatively small, therefore data need to be further tested in larger groups.

keywords: stem cell, encephalopathy, Drug Resistant Epilepsy

94238144705

CHAOTIC PRINTING OF HYDROGEL CARRIERS FOR MESENCHYMAL STEM CELL PROLIFERATION

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Cell-based therapies in the clinic are limited by the number of cells that can be produced quickly and inexpensively. Whereas about one million cells are isolated from a single donor, existing cell-based therapies can require hundreds of millions to billions of cells. Rapid, exponential expansion of cell number would allow faster delivery of life-saving treatments, such as bone marrow transplants, to a greater number of patients.. Chaotic printing is a novel, patent-pending, high-resolution biofabrication technology that could dramatically improve cell expansion capabilities. It produces layered filaments with significantly higher Surface Area per unit Volume (SAV) than existing cell proliferation systems. Cell-laden hydrogel layers can be interspersed with “fugitive ink” layers. The fugitive ink dissolves and evacuates to leave open channels in between the cell-laden layers. The higher SAV should facilitate an exponentially larger interface between nutrient media and cells cultured in these filaments. We predicted this would dramatically improve the speed and yield of cell proliferation by improving nutrient availability and waste removal. In a first experiment, Bone Marrow-derived human Mesenchymal Stem Cells (BM-hMSCs) were cultured for 1 month in chaotically printed hydrogel filaments. To observe the open channels’ impact on cell expansion, hydrogel filaments with alternating cell-laden layers and open channels were compared to a control group of hydrogel filaments without any open channels. The group with open channels had significantly more cells than the control group on days 1, 7, 14, and 21 of culture. The largest difference between the groups occurred on day 7, the open channel group having 2.1 times as many cells as the control group. This experiment showed BM-hMSC viability for 1 month with our hydrogel formulation and chaotic printing method while also demonstrating increased cell expansion rate with open channels present. We also observed a peak expansion rate within the first week with the open channel design. We proceeded to develope and validate a novel bioreactor design for perfusing the open layers in our chaotically printed hydrogel filaments with flowing nutrient media. We hypothesized that adding flow to the system would further improve nutrient availability and waste removal. Co-axially extruding calcium chloride along with the hydrogel bioinks through our printhead allowed for chaotically printed hydrogel filaments to be solidified and extruded directly into small polystyrene tubes. This method results in consistent hydrogel filaments that contain open channels and are flush to the edges of the polystyrene “bioreactor” tubes into which they are extruded. This well-fitting bioreactor system ensures the flow of nutrient media through the open channels. These tubes can then be cultured within an incubator while

connected to a flow circuit driven by a peristaltic pump located outside the incubator. A second experiment demonstrated that BM-hMSCs continued expanding in number for 1 week in chaotically printed hydrogel filaments housed in polystyrene bioreactor tubes when media was flowed through (i.e., replaced via flow) twice per day. Cell-laden filaments in tubes without flow regimens had virtually no viability by day 4. Work is ongoing on a flow regime that will optimize BM-hMSC expansion.

keywords: bioprinting, hydrogels, mesenchymal stem cells, cell expansion, bioreactors

94238119124

TAKING A STEP AHEAD: ENDOCHONDRAL BONE REGENERATION OF A CRITICAL SIZE DEFECT IN A LARGE ANIMAL MODEL

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Endochondral bone regeneration (EBR) recapitulates natural development of long bones during embryogenesis and fracture repair through implantation of a cartilaginous template into a defect, which is eventually remodeled into bone. Typically, autologous multipotent mesenchymal stromal cells (MSCs) are exploited to generate such cartilage constructs. However, the use of patient-own cells is associated with donor-site morbidity and donor-to-donor variability with respect to chondrogenic differentiation potential. Non-autologous MSCs are a promising alternative and offer an off-the-shelf solution due to pre-selection of chondrogenically potent donors, but could possibly cause immune rejection [1]. Previously, we demonstrated that by devitalization of allogeneic cartilage constructs, an adverse immune reaction was avoided, and full defect bridging of a critical size rat femur defect was achieved [2]. The next step to bring this approach into the clinic is the transition from small animal research to pre-clinical studies in large animals. However, successful bone regeneration through EBR using allogeneic constructs has not been achieved in large animal models before. This study aimed at proof of concept of devitalized, allogeneic cartilage constructs for EBR in a critical size defect in a large animal model.

Goat MSCs (gMSCs) were isolated from the iliac crest of Dutch Milk goats, encapsulated within a collagen hydrogel and differentiated in chondrogenic medium for 28 days, followed by devitalization [2]. Constructs were implanted into bilateral, critical size iliac crest bone defects. Each defect was divided into three equal compartments by a titanium spacer. Experimental groups consisted of allogeneic devitalized cartilage constructs. Controls contained gold standard bone autograft, empty carrier control and empty defect (n = 6 for each group). One and two months post-implantation, fluorochromes (calcein and oxytetracycline) were administered intravenously to mark sites of active bone formation at the time of administration. Animals were sacrificed 3 months post-implantation and explanted samples were evaluated for mineralization and new bone formation via microCT, histology, histomorphometry and presence of fluorochromes.

Preliminary results demonstrate the feasibility of EBR using allogeneic cartilage constructs in a large animal model for the first time. Moreover, a critical size defect at the cm3-scale was regenerated by a cell-based implant. Devitalized allogeneic constructs induced more bone formation than the empty (2x more) and carrier (1.5x more) control. Further, the constructs induced new bone formation comparable to the gold standard autograft, as shown by microCT and histological analysis. These findings indicate that our novel approach can achieve bone regeneration, at least comparable to bone autograft, without associated drawbacks such as donor-site morbidity and a second surgical intervention. As this is the first time an allogeneic EBR approach has performed successfully in a pre-clinical large animal model, these results contribute to the clinical translation of EBR in the form of an off-the-shelf product. Further, these results allow us to take the next steps towards clinical translation of our approach by evaluating its safety and efficacy in further pre-clinical models, before proceeding to first in-man trials.

1 Longoni, A. et al., Frontiers in bioengineering and biotechnology 8, 651 (2020).
2 Longoni, A. et al., Advanced science (2021).

keywords: endochondral bone tissue regeneration, devitalization, mesenchymal stroll cells, large animal model, off-the-shelf product

S42
Microphysiological models as powerful preclinical tools
Room: S3 B
(30 Jun 2022, 15:30 - 17:00)

Conveners:
Ozlem Yesil-Celiktas

73387304968

MICROENGINEERING 3D PERFUSION NETWORKS FOR HUMAN LIVER TISSUE MODELS

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Human liver tissue models are of great interest for toxicology analysis in drug development and as liver disease models. All solid tissues depend on sufficient oxygen supply for survival and on controllable oxygen tension for their proper function. Liver tissue models pose a particular challenge in oxygenation given the high oxygen consumption rate of hepatocytes and the well documented gradient in oxygen tension along the liver sinusoid. The oxygen gradient is thought to be key in establishing liver zonation that is required to mimic the in vivo compound metabolism. We have addressed this challenge of reproducing the vasculature function of liver tissue by microengineering massively parallel microfluidic 3D networks in materials open to oxygen- and nutrient-diffusion. The liver-like 3D tissue is cultured between the perfusion channels, thereby shielding the sensitive hepatocytes from shear stresses of the medium flow. In collaborations with cell biologists, the developed technology platform has been employed for the culture of primary human hepatocytes at in vivo-like cell densities for weeks with retained hepatic function as well as culture of human induced pluripotent stem cell-based liver-like cell tissues for months resulting in improved tissue maturation. Gradients in oxygen tensions naturally develop within the cultured tissue due to cellular oxygen consumption, and the cellular oxygen consumption rate depends on the changing local oxygen tension, which makes numerical modeling of the oxygen distribution within tissues highly uncertain without access to a ground truth for validation. We overcome this limitation by development of an optical non-contact method for mapping the actual oxygen concentration in 3D within tissues during culture. The method is based on initial co-seeding of tissue cells with oxygen sensing microbeads in the culture platforms and readout of the oxygen distribution using confocal phosphorescence lifetime microscopy (PLIM).

keywords: Synthetic vasculature, oxygen supply, stereolithography, 3D printing, liver models

62903407479

DESIGN AND FABRICATION OF AN ORGAN-ON-A-CHIP TECHNOLOGY AS A PHYSIOLOGICALLY RELEVANT IN VITRO MODEL OF THE OUTER BLOOD-RETINAL BARRIER

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Age-related macular degeneration (AMD) is a chronic eye disease and the leading cause of irreversible vision loss in millions of elderly people world-wide. The ocular system implicated in this disease is the outer blood-retinal barrier (oBRB) comprised of the retinal pigment epithelium (RPE), the Bruch's membrane (BrM), and the choriocapillaris (CC). The oBRB plays a pivotal role in maintaining the eye homeostasis by regulating the transport of nutrients and metabolic wastes from choroid to the sub-retinal space. In AMD patients, several morphological and structural changes occur in the oBRB resulting in its disfunction and a failure of such homeostasis. At present, due to the multifactorial nature of the AMD, the exact disease pathogenesis remains poorly understood. As a result, although palliative cares exist for only some forms of AMD, no effective treatments exist. In order to investigate the pathophysiological process underlying AMD and to validate novel drug candidates, several in vivo and in vitro models have been proposed. However, none of these have proven to be reliable to mimic the complex cellular interactions in the oBRB with physiological realism and great predictive value. Therefore, herein we present a novel oBRB-on-a-chip model as a biomimetic platform for AMD understanding and for new therapeutic agents development. The device is a 3D microfluidic platform consisting of a biomimetic blood vessel network mimicking the CC and of a novel BrM-mimetic bio-membrane both housed within a single-chamber which resembles the intraocular space and enables the co-culture of human RPE and endothelial cells above the BrM and inside the CC respectively. The microfluidic network, designed starting from Optical Coherence Tomography (OCT) scans , was fabricated from polydimethylsiloxane (PDMS) through a novel manufacturing method established to provide a time-saving and cost-effective alternative to the common lithographic-based techniques. The interior surfaces of the microfluidic channels were subsequently coated with chemically crosslinked gelatin to promote cell adhesion and long-term culture. The engineered BrM was fabricated from chemically crosslinked gelatin by electrospinning process to get porous, ultrathin and nanofibrous membranes mimicking the mechanical, chemical and physical properties of the native substrate. The co-culture chamber with a common internal footprint with the wells in standard 24-well plates was fabricated from PDMS via demoulding process. Perfusion tests were successfully performed for validating the overall microfluidic platform. Human embryonic stem cell-derived RPE cells and HUVECs cells were cultured on the engineered BrM and on PDMS-gelatin substrates respectively to evaluate cells adhesion and proliferation under static conditions. Immunofluorescence microscopy demonstrated that engineered BrMs supported functional RPE monolayer formation, while HUVECs cells shown good adhesion and proliferation on the PDMS-gelatin substrates. Tests of dynamic seeding and static/dynamic co-culture of the cellular species involved are in progress to set out standard protocols. Taken together our findings are encouraging, showing that we successfully designed

a physiologically relevant oBRB model to study AMD in vitro, in which patient-derived cells could be used for the identification new drugs paving the way towards personalized medicine.

keywords: Biofabrication, in vitro model, biomaterials, bioengineered platform, Age-related macular degeneration

20941827909

A GUT-BRAIN AXIS PLATFORM TO MODEL BRAIN FLUIDS CLEARANCE IN NEUROINFLAMMATION

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Introduction
Gut microbiota is able to communicate with the brain through complex bidirectional routes constituting the so-called microbiota-gut-brain axis (MGBA). Gut microbial dysbiosis increases local inflammation also thanks to the secretion of bacterial lipopolysaccharides (LPS), which disturbs the gastrointestinal and also the blood-brain barrier (BBB) permeability thus propagating inflammation to the brain 1. A fluid-clearance pathway in the brain, the glymphatic system (GS), was recently discovered with increasing evidence of its role in neurodegeneration by controlling the clearance of neurotoxic proteins through convective exchange of flows 2.
An in vitro model of GS in the scenario of neuroinflammation is missing. Here, we developed a GS model based on tunable levels of brain fluids clearance in a 3D brainlike environment, to be integrated in a MGBA engineered multiorgan-on-a-chip platform developed within the ERC project "MINERVA", which aims at studying the intestinal microflora impact on brain functionality 3. The presence of a GS model in the brain unit of the multiorgan platform, combined with gut epithelial and BBB on-a-chip systems will allow to recapitulate LPS-mediated neuroinflammation in the presence of pathophysiological brain fluids drainage representing an innovative tool to study MGBA and GS role in dementia.

Methodology
The hydrogel based 3D brain model was engineered starting from a previously validated formulation 4.
Interstitial flow levels and molecules transport within the 3D brain model were predicted by multiphysics computational analysis and subsequently validated by measuring dextran molecules transport at different molecular weights (4, 40 and 70 kDa). The biological validation was performed by measuring cell viability for the cell models used to model the gut and BBB barriers as well as brain inflammation: intestinal epithelial cells (CaCo2); endothelial cells (bEND.3); astrocytes (C8D1A); neuroglioma cells (H4). Transepithelial Electrical Reistance (TEER) was measured to evaluate gut epithelial and BBB barriers integrity. Fluorescent LPS was tracked along the platform by fluorescence intensity detection. Interleukin 6 (IL-6) production by H4 cells at different fluid clearance levels was measured by ELISA.

Results
Computational analysis predicted flow velocities profile inside the 3D matrix and a physiological range of 1-10 uL/min was selected with clearance velocities corresponding to 0.14-1 um/s.

Solutes clearance was validated with higher molecular wheight molecules being entrapped inside the hydrogel at low level of flows. The platform sustained the long-term culture of all cells models.
After LPS stimulation of the barrier models, TEER values decreased and IL-6 detectable levels inside the 3D brain model were measured. Preliminary results showed IL-6 production being modulated at different clearance flows levels suggesting an active role of fluid drainage in controlling neuroinflammatory response.

Conclusions
The integration of GS in a MGBA engineered multiorgan platform represents a suitable tool for modelling in vitro both physiological and pathological drainage of fluids inside the brain by recreating tuned GS interstitial flows within MGBA inflammation models.

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keywords: neurodegeneration, glymphatic system, 3D model, brain-on-a-chip, microbiota-gut-brain axis

31412716105

PRELIMINARY DEVELOPMENT OF AN IN VITRO 3D IPSC-BASED LIVER MODEL TO EXPLOIT AN INNOVATIVE LIVER-ON-A-CHIP DEVICE

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Introduction
Organ-on-a-chip (OoC) technology shows great potential to accelerate drug discovery and advance personalized medicine. Induced pluripotent stem cells (iPSCs) may enhance the predictivity of the OoC in the assessment of patient's response to a pharmacological treatment and related toxicities. Nowadays, there are no body-on-a-chip fully based on iPSCs used for drug screening. In this context, our PEGASO project aims to develop the first iPSC-based multiorgan-on-chip for Alzheimer's disease drug development. The full platform will be composed of six OoC devices connected and loaded with iPSCs-derived models representing the main body systems involved in dementia drug pharmacokinetics (microbiota/gut, immune system, liver, blood-brain-barrier and brain tissue). Here, we present preliminary characterization of an in vitro 3D iPSCs-based liver model to be hosted into our innovative PEGASO OoC device.

Methodology
An in vitro 3D model with commercially available human iPSC-derived hepatocytes and endothelial cells has been chosen as a liver tissue model for the dynamic culture inside the PEGASO OoC, where hepatocytes encapsulated into a collagen-poly(ethylene)glycol hydrogel and endothelial cells have been cultured interconnected once seeded on each side of a porous membrane hosted into the OoC.
A 2D model with seeded membrane was used as control. Cell viability was assessed with MTS assay while albumin and urea produced by hepatocytes with ELISA and urea colorimetric assay respectively.
The mRNA expression level of CYP3A4 in hepatocytes was evaluated with RT-PCR while the protein expression of albumin and HNF4α with Western Blot and immunofluorescence.
The shear stress and oxygen concentration to which the cells are exposed under dynamic conditions have been assessed by a computational model developed with COMSOL Multiphysics ®.

Results
Hepatocytes grown inside the hydrogel 7 days after plating exhibited comparable metabolic activity with cells in 2D control condition. Differently, the liver-specific functions, referred to albumin and urea synthesis, resulted significantly higher in the 3D model with respect to the control. The protein expression of albumin and HNF4α, that are key hepatocytes markers, analysed with Western Blot and immunofluorescence was increased in the 3D condition.
The expression of CYP3A4 indicated a higher detoxification ability by hepatocytes in 3D condition with respect to the control.
To select the optimal flow rate for the dynamic culture of the iPSC-derived liver in our organ-on-a-chip, a computational simulation was performed with the software COMSOL Multiphysics ®,

tailoring the model for hepatocytes specific requirements, that are shear stresses <0.2 Pa and oxygen consumption of 0.3 nmol/s*m3[1,2].
The numerical simulation indicated 30 µl/min as the proper medium flow rate, leading to adequate shear stresses (range 0.01-0.03 mPa) and oxygen concentrations (range 0.18-0.2 mol/m3).

Conclusions
Taken together, the biological and computational results suggest that our 3D liver model is a suitable iPSC-derived model to be hosted and cultured under perfusion in the PEGASO OoC.

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keywords: induced pluripotent stem cells, liver, organ-on-a-chip, 3D

52354536639

A MICROFLUIDIC PLATFORM TO INVESTIGATE THE CROSS-TALK BETWEEN IMMUNE CELLS IN RHEUMATOID ARTHRITIS

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INTRODUCTION
Rheumatoid arthritis (RA) is an autoimmune disease that affects diarthrodial joints, characterized by a systemic inflammatory response that leads to progressive joint destruction [1]. Various immune cells are involved, but the exact mechanism of RA development is still unknown, and it is well established that none of the currently available animal models fully represent human RA onset and progression [2]. In this scenario, organs-on-chip provide a valuable solution to evaluate the involvement and the interaction of different cell types in RA conditions. To this aim, we developed a novel microfluidic platform allowing to study the cross-talk between immune cells involved in RA.

METHODOLOGY
The platform consists of two separate culture areas, whose communication is controlled through normally closed valves. The first compartment is intended to host cells seeded in a 3D matrix, e.g. macrophages, while the second one is composed of a single channel designed to precisely locate and culture cells in suspension, e.g. T cells. The second compartment encompasses a novel technology, named “sieving valve”, which relies on normally closed valves with underneath microfluidic channels that allow perfusion of fluids but impair cell escaping when closed. CD14+ macrophages and CD4+ T cells isolated from human buffy coat were independently seeded in the two separate compartments and stimulated with TNFa and IL15, respectively. Upon central valve opening, recruitment of CXCR6+ T cells operated by inflamed macrophages expressing CXCL16 was evaluated [3].

RESULTS
A protocol was first optimized to seed and trap CD4+ T cells inside the microfluidic platform, taking advantage of the sieving valve technology. Cells were then successfully stimulated inside the platform exploiting the separation of the compartments. As proven by immunofluorescence staining, CXCL16 expression was enhanced in macrophages after treatment with TNF-α, while CXCR6 expression was up-regulated in T-cells after stimulation with IL-15. After stimulation, migration of T-cells towards macrophages occurred spontaneously upon opening of the communication valves, as quantified through live imaging.

CONCLUSIONS
The proposed device offers an innovative solution to trap immune cells inside microfluidic chips and to study the cross-talk between different cell types, having the possibility to stimulate them separately. The platform was validated replicating a known mechanism in RA, involving resident macrophages and T cells [3]. The use of the platform to elucidate the role of RA-patient specific circulating immune cells on synovial membrane is currently under evaluation, and will eventually increase the understanding about unknown mechanisms in RA progression. Moreover, the technology is highly versatile and can be potentially applied to assess the interaction of immune system in manifold diseases and more complex models.

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ACKNOWLEDGEMEBNTS
We thank Dr. Carlotta Catozzi for her technical help in the experiments. This work was supported by Fondazione Cariplo-uKNEEque - Rif. 2018/0551 and has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 841975.

keywords: microfluidics, organs-on-chip, rheumatoid arthritis, immune system, immune cells

73296325005

A TUNABLE LUNG PHYSIOMIMETIC STRETCH SYSTEM EVALUATED WITH PRECISION CUT LUNGS SLICES AND RECELLULARIZED HUMAN LUNG SCAFFOLDS

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Breathing exposes lung cells to continual mechanical stimuli, which is part of the microenvironmental signals directing cellular functions. Therefore, developing in vitro model systems that incorporate physiomimetic mechanical stimuli is urgent to fully understand cell behavior. This study aims to introduce a novel in vitro culture methodology combining mechanical stimuli that simulates in vivo breathing in 3D cell culture platforms in the form of recellularized human lung extracellular matrix (ECM) scaffolds and precision cut lung slices (PCLS) from rats.

To this end, we have constructed a device for controlled cyclic stretch, mimicking amplitudes and frequencies of distensions seen in the breathing human lung. For its validation, we cultured H441 lung epithelial cells in decellularized human lung slices exposed to 16 stretch cycles per minute with a 10% stretch amplitude. Cell viability (resazurin reduction), proliferation (Ki-67) and YAP1 activation were evaluated at 24 and 96 hours by immunohistochemistry, while the expression of SFTPB, COL3A1, COL4A3 and LAMA5 was evaluated by qPCR.

Cyclic stretch induced an increase in SFTPB expression after 24 hours without a concomitant increase in the stretch responsive gene YAP1. The ECM milieu lowered the expression of the basement membrane protein genes COL4A3 and LAMA5 compared to tissue culture plastic control cultures, without any additional effect induced by the mechanical stimuli. Additionally, we show compatibility of the device with PCLS culture showing preserved morphology and metabolism in rat PCLS after 72 hours of mechanical stretch. Thus, we present a novel device and methodology for the study of lung tissue slice cultures subjected to physiomimetic mechanical stimuli, which shows promise for future studies of cell and tissue function in a lung ECM milieu with physiological or pathological mechanical stimuli.

keywords: lung physiomimetic model, mechanical stimuli, decellularized extracellular matrix, stretch, 3D

31412745855

SPATIO-TEMPORAL EMERGENCE OF MULTICELLULAR ENGINEERED STRUCTURES AS PRECLINICAL MODELS

Ozlem Yesil-Celiktas (Ege University, Izmir, Turkey), Pelin Saglam-Metiner (Ege University, Izmir, Turkey)

Multicellular tissues such as spheroids, organoids can be assembled in vitro from clusters of cells in a spatio-temporal manner, mimicking in vivo physiology and tissue microenvironment, which can leverage pre-clinical evaluation of the efficacy and safety of potential new drugs for diseases such as hereditary, neurodevelopmental, infectious and cancer. In one approach, immortal, primary or differentiated pluripotent cells are seeded in three-dimensional (3D) constructs leading to assembled multicellular structures, whereas the other approach involves the self-organization of cells that are subjected to a variety of external stimuli such as physical, chemical, mechanical and electrical. One of the greatest challenges is the regulation of spatio-temporal organization and/or differentiation of cells within a 3D microenvironment. In this study, the impact of microgravity in self-assembly of cells was evaluated in a bioengineered system that provided controlled dynamic flow conditions, supported growth factor diffusion, metabolite exchange in larger sizes and survival. The formation of multicellular structures was not only investigated on cellular level including morphology, proliferation, adhesion but also on functional level in terms of recapitulating the physiology. Reproducible scaling of these engineered spheroids and organoids in consecutive batches will allow high-throughput screening of emerging therapeutics and combinational therapies as preclinical models at an industrial scale, which is envisaged to accelerate in the near future.

The financial support provided by TUBITAK through grant no. 120N422 is highly appreciated.

keywords: multicellular systems; organ-on-chip; disease modeling; high-throughput screen; therapeutics

S43-1
Multifunctional biomaterials supporting bone regeneration
Room: S2
(29 Jun 2022, 11:00 - 12:30)

Conveners: Timothy Douglas; Elżbieta Pamuła

41935603124

CURRENT STATUS AND FUTURE PROSPECTS OF GENOME-SCALE METABOLIC MODELING TO OPTIMIZE THE USE OF MESENCHYMAL STEM CELLS IN REGENERATIVE MEDICINE

Olafur Sigurjonsson (Reykjavik University, Reykjavik, Iceland)

Mesenchymal stem cells are a promising source for externally grown tissue replacements and patient-specific immunomodulatory treatments. This promise has not yet been fulfilled in part due to production scaling issues and the need to maintain the correct phenotype after re-implantation. One aspect of extracorporeal growth that may be manipulated to optimise cell growth and differentiation is metabolism. The metabolism of MSCs changes during and in response to differentiation and immunomodulatory changes. MSC metabolism may be linked to functional differences but how this occurs and influences MSC function remains unclear. Understanding how MSC metabolism relates to cell function is however important as metabolite availability and environmental circumstances in the body may affect the success of implantation. Genome-scale constraint based metabolic modelling can be used as a tool to fill gaps in knowledge of MSC metabolism, acting as a framework to integrate and understand various data types (e.g., genomic, transcriptomic and metabolomic). These approaches have long been used to optimise the growth and productivity of bacterial production systems and are being increasingly used to provide insights into human health research. Production of tissue for implantation using MSCs requires both optimised production of cell mass and the understanding of the patient and phenotype specific metabolic situation. This presentation will discuss the current knowledge of MSC metabolism and how it may be optimised along with the current and future uses of genome scale constraint based metabolic modeling to further this aim.

keywords: Metabolomics, Stem cells, Omics, Modeling

94238145306

OSTEOINDUCTIVE INJECTABLE CALCIUM PHOSPHATE BIOACTIVATED BY PHOSPHOSERINE DENDRONS

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Introduction
In recent years, the clinical application of injectable bone pastes has been increasing because of their benefits including ease to adaptation into irregular-structured defects and injectability suitable to minimally invasive surgery. These advantages are widely advocated to reduce patient complications and health care costs. Here, we successfully developed injectable bone pastes integrating biphasic calcium phosphate (BCP, HA:β-TCP=1:4) nanoparticles with poly(-lysine) dendron activated by phosphoserine at generation 3 (G3-K PS). Furthermore, the incorporation of strontium (Sr) element into the BCP nanocrystals was also considered to minimize bone resorption.

Methodology
The preparation of materials including G3-K PS semi-dendrimers, BCP, SrBCP (with 15 mol% Ca2+ replaced by Sr2+), BCPG3 (BCP in G3-K PS carrier) and SrBCPG3 (SrBCP in G3-K PS carrier) was adapted from previously protocols1. G3-K PS semi-dendrimer in ethanol solution was added at 1% (w/w) to BCP or SrBCP slurry under 200 rpm and 37 °C stirring until gelation occurred. In vitro cell tests to assess the osteogenic potential of the synthesized biomaterials were performed using bone marrow stromal cells (MSCs) isolated from ovariectomized rats and the expression of specific gene markers (Runx2, ALP, Cxcl9, RANKL) at 1, 3 and 7 days of cell culture were analyzed by RT-PCR. To confirm the role of Cxcl9 and Sr ions in material induced osteogenesis, additional Cxcl9 protein (250 ng mL-1) was introduced into the BCPG3 and SrBCP groups in culture medium, and Sr ions (3 mM) were introduced into the BCP and BCPG3 groups in cell culture medium. In vivo studies were performed using ovariectomized female Sprague Dawley rats. Several bone metabolic markers including P1NP, CTX-I and Cxcl9 were evaluated using ELISA assay at week 12. Immunohistochemistry analysis, micro-CT and scanning electron microscopy investigations were performed to evaluate the new bone tissue formation and osteointegration.

Results
Injectable bone paste material integrating BCP nanoparticles with G3-K PS carrier was successfully synthesized with or without the doping of Sr element into the BCP nanocrystals. Both in vitro and in vivo findings showed that the integration of G3-K PS would downregulate Cxcl9 gene and protein expressions to achieve an enhanced bone regeneration effect, with respect to a higher BMD and BV/TV. Immunohistological staining demonstrated lower expression of Cxcl9 in BCPG3 and SrBCPG3 groups, meanwhile Runx2 and RANKL positive expressions were more in BCPG3 group than others. The results were in accordance with in vitro gene and protein expression and in vivo serum biomarker analysis. This study demonstrated for the first time that G3-K PS carrier could down-regulate Cxcl9 expression and no additional benefit to osteoporotic bone regenerating ability of BCPG3 material was found with Sr incorporation.

Conclusion
The results indicated that the BCPG3 bone paste can become a high-performance bone filler in the treatment of osteoporotic bone defects.

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keywords: injectable biomaterials, dendrons, bone regeneration, in vitro cell-material interaction, in vivo model

73296331205

MECHANICAL STIMULATION PROMOTES THE OSTEOGENIC RESPONSE OF PRE-OSTEOBLASTS ON POLYMERIC SCAFFOLDS

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Introduction
Bone is a highly dynamic tissue that undergoes continuous remodeling through lifetime. During this process, two cell types, osteoblasts and osteoclasts, are responsible for bone formation and bone resorption respectively. Mechanical stimuli applied on bone tissue can shift the balance between these two cell populations [1]. Different studies have investigated the impact of the mechanical stimuli on cell proliferation and differentiation [2, 3]. Pre-osteoblastic cells are well established to evaluate in vitro osteogenic responses [4], and they can differentiate into mature osteoblasts under appropriate culture conditions, allowing for the validation of bone tissue formation. In this study, the application of uniaxial compression on pre-osteoblastic cells seeded onto PLLA/PCL/PHBV scaffolds was examined by assessment of cell viability and differentiation markers to determine the effect of the mechanical stimulation on cellular responses.

Methodology
MC3T3-E1 pre-osteoblastic cells (7x104 cells/scaffold) were seeded onto blend scaffolds (5 mm x 5 mm x 1 mm) consisting of PLLA/PCL/PHBV (90/5/5) and cultured under both dynamic and static conditions for 21 days. The scaffolds were subjected to mechanical stimulation for 40 min every day. Three different frequencies of uniaxial compression, 0.5, 1, and 1.5 Hz, with a strain equal to 8% of the scaffold side (400 μm of displacement), were employed to assess their effect on pre-osteoblasts' differentiation. Cell proliferation and morphology were monitored via a cell viability assay and scanning electron microscopy (SEM). Measurement of alkaline phosphatase (ALP) activity and calcium secretion were conducted to determine the effect of the mechanical stimulation on the osteogenic responses of pre-osteoblasts, and were compared to the static condition.

Results
All dynamic conditions depicted lower cell number than the static equivalent, however comparable at all experimental time periods. These finding are in line with the SEM images showing that the cells adhered strongly to the scaffolds from the early experimental time points. The ALP activity in all dynamic conditions demonstrated significantly higher values ($p<0.0001$ for 0.5 Hz, and $p<0.001$ for 1 and 1.5 Hz) than those of the static one on Day 7. Similarly, the calcium secretion by pre-osteoblasts demonstrated the highest values in all dynamic conditions on Day 7, with the condition at 0.5 Hz indicating the highest level, followed by those at 1 and 1.5 Hz. At

Day 14, the calcium concentration decreased, while at Day 21 all conditions displayed similar levels to Day 14.

Conclusions
This study demonstrated that the applied mechanical stimuli affected the cell viability and osteogenic differentiation of the pre-osteoblastic cells leading to their differentiation into mature osteoblasts, revealing that the most efficient applied stimuli condition was at 0.5 Hz.
This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 814410.

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keywords: mechanical stimulation, dynamic vs static cells culture, uniaxial compression, polymeric scaffolds, osteogenic differentiation

83767247288

BIOFABRICATION OF THE VASCULARISED OSTEOGENIC NICHE

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The establishment of vascularisation is one of the current challenges, especially in critically sized bone defects [1,2]. Therefore, the aim of the present work is to develop a biomimetic hydrogel scaffold mimicking the bone osteoid in which proper vasculature is induced in order to maximally stimulate osteogenic regeneration. Thiol-ene step-growth photocrosslinking was herein selected to enable superior control, homogeneous network formation and lower radical concentrations compared to traditional chain-growth systems [3]. The networks were benchmarked against traditional chain-growth systems in a physico-chemical and an in vitro osteo- and angiogenic evaluation.

For osteogenic purposes, a novel norbornene-modified aminated gelatin was developed with a degree of substitution of 135% compared to the amount of amines present in native gelatin type B (by addition of only 1.2 equivalents of norbornene carboxylic acid). It is, to the best of our knowledge, the highest substitution reported for norbornene-functionalised gelatins [4]. Thiol-ene crosslinking with thiolated gelatin as cell-interactive crosslinker resulted in networks with full norbornene conversion and a gel fraction of 99%. The increase in storage modulus compared to non-aminated thiol-ene and gelatin-methacryloyl systems (through reaction with the same amount of equivalents) was attributed to an increase in chemical crosslinking (1.8-fold and 4.6-fold respectively) and a decrease of swelling (3.2-fold and 2.9-fold). The biodegradation properties of this hydrogel were preserved and direct contact cell viability data indicated an extended morphology and excellent biocompatibility (96% viability after 7 days). Osteogenic differentiation seeding tests indicated an increase in alkaline phosphatase (ALP) production after 7 days (2.8-fold and 1.5-fold) and a rise in calcium deposition after 21 days (5.2-fold and 1.7-fold). In addition, the incorporation of amorphous calcium phosphate into the thiol-ene hydrogel further contributed to the osteogenicity (1.1-fold increase in ALP-production and 1.2-fold increase in stem cell-based calcium deposits).

Thiol-ene systems were also benchmarked against chain-growth systems with regard to angiogenic stimulation. The homogeneous networks showed similar attachment, yet a more pronounced vascular signalling capacity in terms of fibroblast growth factor-1 (1.7-fold increase after 1 day) and epidermal growth factor (1.2-fold increase after 1 day) secretion compared to the more heterogeneous chain-growth networks which signalled mainly through the production

of placental growth factor (2-fold increase after 1 day). Moreover, the thiol-ene networks showed an increased seeded endothelial cell viability (1.2-fold after 7 days) and metabolic activity (1.3-fold after 7 days). The incorporation of human placenta substrate to the thiol-ene networks greatly stimulated the sprout junction density, the total tube length and the number of branches within the developed vascular network in these hydrogels.

The results show that highly controlled networks could be created with tailored topology and viscoelastic behaviour which can stimulate angio- and osteogenesis. Ongoing work focusses on the processing of these hydrogels as bio-inks through extrusion-based 3D-printing and evaluation of the 3D-printed constructs towards vascularised osteogenesis.

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keywords: BIOMATERIALS – BONE TISSUE ENGINEERING – PHOTO-CROSSLINKABLE HYDROGELS - VASCULARISATION

31412759499

CALCIUM PHOSPHATE BASED BIOMATERIALS INFLUENCE ON CELL METABOLISM

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INTRODUCTION
Understanding the interaction between cells and materials is fundamental to tissue engineering. The scientific community's focus on biomaterial-cell interactions has largely fallen on cellular phenotypes, proteins, and nucleic acids, while molecular mechanisms within cells are often overlooked. However, it is expected that cellular metabolism will be significantly influenced by biomaterials. In this regard, direct measurement of metabolites can elucidate perturbation in cellular metabolism, helping to explain and predict changes in cellular phenotypes. In this work, we performed in vitro based metabolomics study to identify changes in cellular metabolism upon interaction with calcium phosphate based biomaterials.

METHODS
Calcium phosphates, including hydroxyapatite (HAP), β-tricalcium phosphate (β-TCP), and bicalcium phosphate (BCP), were applied as the model materials to investigate the cellular response. Sintered materials were characterized by XRD to determine the crystalline structure. NIH/3T3 cells were directly seeded on the materials and harvested on days 1, 3, 5, and 7, following methanol-chloroform mixture extraction protocol to collect metabolites and lipids. Targeted quantitative metabolite analysis was carried out utilizing several HPLC methods in combination with mass spectrometric detection. Obtained metabolomics data were further analysed using MetaboAnalyst 5.0.

RESULTS
The results showed that different calcium phosphate components induced different changes in cellular metabolism. Pathway analysis comparing metabolites profiles between cells grown on HAP, β-TCP, and BCP from different time points and control cell culture showed that metabolism and biosynthesis of several amino acids, acylcarnitines, and lipids were changed. For example, we observed changes in glutathione and arginine metabolism which plays important roles in antioxidant defence and immune response. The observed changes in amino acid metabolism were inter-interpretable with previously reported gene expression and protein activity data for selected materials.

CONCLUSIONS
In our study, we confirmed the calcium phosphate based biomaterials influence on cell metabolism. Distinct changes in metabolite profiles for cells seeded on calcium phosphate ceramics compared to controls were observed. Most of the detected metabolites exhibited time-dependent changes indicating a cellular adaption mechanism to biomaterial-induced perturbation. These results provide evidence about the biomaterials chemical composition influence on cellular metabolism and its link to gene expression level. This proves that metabolomics is a useful tool for studying metabolomics-material interactions to improve the current understanding of the mechanism governing cell behaviours.

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keywords: Interactions, Metabolomics, Calcium phosphate, Metabolism

83767259297

EVALUATION OF Β TRICALCIUM PHOSPHATE AND POLY(3-HYDROXYBUTYRATE) -BASED SCAFFOLDS FOR BONE TISSUE REGENERATION

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Tissue engineering proposes an innovative therapeutic approach to support and induce regenerative processes in damaged tissues. Scaffolds should provide a suitable environment for proliferation, differentiation, and maturation of cells and formation of new tissue and blood vessels. Furthermore, scaffolds should be gradually resorbed at a rate commensurate with bone formation. Calcium phosphates (CaPs) are commonly used in bone tissue engineering due to their chemical composition similarity to the main component of the inorganic part of bone. Among CaPs, β tricalcium phosphate (β-TCP) exhibits a suitable resorption rate for tissue engineering applications [1]. However, highly porous ceramics demonstrate high brittleness and poor surgical handling. Polymeric coatings tend to improve the durability of bioceramic scaffolds and may serve as carriers of biologically active substances [2]. Poly(3-hydroxybutyrate) (P(3HB)) is a biocompatible and biodegradable biopolymer, which degradation products are harmless to the surrounding tissues. For these reasons, we used P(3HB) as a coating on β-TCP scaffolds. The physicochemical and biological properties of the obtained materials have been examined.

β-TCP scaffolds were fabricated by a foam replication method using three types of polyurethane matrices with different pore sizes (S-small, M-medium, L-large). The bioceramic scaffolds were soaked in the P(3HB) solution, dried, and subjected to further studies. Obtained materials were assessed by X-ray diffraction, scanning electron microscopy, hydrostatic weighing, compression tests, and chemical stability in vitro. Degradation products of P(3HB) were analysed via UHPLC-MS. Furthermore, hMSC adhesion, growth, and differentiation were assessed.

The ceramic scaffolds were uniformly covered with the biopolymer, which was evidenced by SEM observations. The P(3HB) coating did not significantly influence the total porosity of the materials obtained (Ptotal~70 vol%). Composites possessed higher comprehensive strength (up to 4.5 ± 0.5 MPa) compared to uncoated β-TCP. The degradation of P(3HB) during incubation in water was confirmed by UHPLC-MS as (R)-3-hydroxybutyric acid and its oligomers were identified in the extracts. In vitro studies revealed that hMSCs adhere, grow, and proliferate on both uncoated and coated scaffolds (viability over 85% at 7 and 21 days). The number of cells increased in day 21 if compared to day 7 on all materials. The pore size affected the depth of penetration of the cells. The cells efficiently penetrate the materials (even 650-700 μm into the scaffold with large pores).

P(3HB) can serve as a coating on ceramic-polymer composites improving mechanical properties and the durability of the scaffolds. In addition, the released (R)-3-hydroxyacids may nourish the surrounding tissues. Preliminary in vitro studies using hMSC revealed no cytotoxicity of β-TCP as well as β-TCP/P(3HB) scaffolds. Developed materials can act as scaffolds for bone tissue regeneration. Further in vivo studies are necessary.

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keywords: Calcium phosphates, Scaffolds, Polyhydroxyalkanoates, Composites

52354527524

OSTEOGENIC ACTIVITY OF ADDITIVE MANUFACTURED TITANIUM ALLOY-CALCIUM PHOSPHATE CERAMIC SCAFFOLDS FOR CRANIOPLASTY IN VITRO AND IN A LARGE ANIMAL CALVARIAL DEFECT MODEL

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Surgical repair of large skull bone defects is often performed using patient-specific metallic implants.(1) These implants, however, have poor osseointegration ability, and their life-long fixation is dependent on osteosynthesis screws.(2) It is often suggested that a composite model bone graft substitute could be used to overcome these problems, combining desired properties from individual material classes.(3) We recently developed new materials that combine the mechanical properties of a titanium alloy (Ti6Al4V) with the bioactivity of a calcium phosphate ceramic (CaP) using additive manufacturing, resulting in porous Ti6Al4V 3D implants without or containing 5 or 10 wt% beta-tricalcium phosphate (TCP).(4) The osteogenic differentiation and tissue formation capacity of bone marrow-derived human mesenchymal stromal cells (hMSCs) on these implants were examined in vitro, and the implants were subsequently tested in vivo in a large animal calvarial defect model.

Standardized cylindrical implants (diameter 5 mm, height 2 mm) were seeded with hMSCs and cultured in basic or mineralization medium. On days 14 and 28, the constructs were analyzed for cell metabolic activity, DNA content, tissue formation, alkaline phosphatase (ALP) activity, osteopontin and osteocalcin secretion, and osteogenic gene expression. Next, critical-size defects were made in the frontal bone of skeletally mature minipigs. Test or control scaffolds (diameter 15 mm, height 5 mm) were used for reconstruction, or the defect was left untreated. The animals were sacrificed after 12 weeks, and the implants were retrieved and analyzed for new bone formation and bone ingrowth.

hMSCs cultured on the scaffolds in vitro remained metabolically active and showed a similar proliferation profile on all scaffold types. hMSCs produced ALP, osteopontin and osteocalcin. Additionally, we observed tissue formation throughout the porous scaffolds in all conditions after 14 and 28 days. RUNX2 and ALP expression showed an inverted relationship with increasing TCP content, whereas osteocalcin and osteopontin were more expressed with increasing TCP content. A detailed analysis of the in vivo results of new bone formation and bone ingrowth into the porous structure is ongoing and results will be summarized in the presentation. Overall, the implants showed a good intra-operative handleability and no complications regarding the implant site were observed.

We have shown successful cell survival and tissue formation on our newly developed Ti6Al4V-TCP scaffold types in vitro. Bone formation and bone ingrowth into the 3D porous implant

structure in the physiologically complex minipig model will enhance our understanding of the effect of the individual components and structural characteristics on the biological response.

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keywords: bone regeneration, titanium alloy, calcium phosphate ceramic, additive manufacturing, 3D porous scaffold

S43-2
Multifunctional biomaterials supporting bone regeneration
Room: S2
(29 Jun 2022, 13:30 - 15:00)

Conveners: Timothy Douglas; Elżbieta Pamuła

62825408706

CONTROLLED DELIVERY OF EPIGENETICALLY ACTIVATED EXTRACELLULAR VESICLES FROM A GELMA/NANOCLAY HYDROGEL FOR BONE REGENERATION

Kenny Man (University of Birmingham, Birmingham, United Kingdom)

Extracellular vesicles (EVs) have garnered growing attention as promising acellular tools for bone repair. Epigenetic regulation through histone deacetylase (HDAC) inhibition has been shown to increase differentiation capacity. Although EVs efficacy has been shown, their short half-life in vivo hinders their therapeutic potency. Gelatin methacryloyl (GelMA) hydrogels functionalised with synthetic nanoclays have been demonstrated to improve growth factor retention. This study investigated the potential of combining epigenetically activated osteoblast-derived EVs with the GelMA nanocomposite hydrogel to stimulate bone repair.

GelMA/nanoclay composites were fabricated by combining 5wt% GelMA with different concentrations of LAP (0.5, 1 and 2 wt%) prior to visible light crosslinking. The hydrogels compressive modulus, shear-thinning behaviour, 3D printing fidelity and osteogenic potency was evaluated. EVs were derived from 5 nM TSA-treated or untreated osteoblasts over a 2-week period. The EVs size, morphology and concentration were assessed via nanoflow cytometry and transmission electron microscopy. The isolated EVs were incorporated within the composites and their release kinetics were determined using the CD63 ELISA. The osteogenic differentiation of human bone marrow stromal cells (hBMSCs) within the EV-functionalised hydrogel was evaluated by qPCR, biochemistry and histological analysis.

LAP improved GelMA compressive modulus and shear-thinning properties in a dose-dependent manner. Nanoclay incorporation enhanced the shape fidelity when 3D printed compared to LAP-free gels. Interestingly, GelMA hydrogels containing LAP exhibited increased mineralisation capacity (1.41-fold) over 14 days. EV release kinetics from these nanocomposites were strongly influenced by LAP concentration with significantly more vesicles released from LAP-free constructs. EVs derived from TSA-treated osteoblasts (TSA-EVs) enhanced proliferation (1.09-fold), migration (1.83-fold), and mineralisation (1.87-fold) of hBMSCs when released from the GelMA-LAP hydrogel compared to the untreated EV gels. Importantly, the TSA-EV functionalised GelMA-LAP hydrogel significantly promoted encapsulated hBMSCs extracellular matrix collagen production (≥1.3-fold) and mineralisation (≥1.78-fold) in a dose-dependent manner compared to untreated EV constructs.

Taken together, these findings demonstrate the potential of combining epigenetically-activated osteoblast-derived EVs with a nanocomposite photocurable hydrogel to enhance the therapeutic efficacy of acellular vesicle approaches for bone regeneration.

keywords: Epigenetics, extracellular vesicles, bone, hydrogel, controlled release

94238118639

BONE REGENERATION OF A CRITICAL-SIZED DEFECT IN SHEEP WITH A 3D PRINTED SCAFFOLD COATED WITH A BIOMETIC FILM CONTAINING LOW-DOSE OF BMP-2

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Introduction
Large bone defects are a challenge for orthopedic surgeons. BMPs are the most potent bone inductors and accelerators of bone growth. However, poor BMP-2 retention by scaffolds leads to its rapid clearance from implantation sites,1 and thus, require the use of supra-physiological doses. Serious concerns regarding BMP-2-related adverse effects have been reported (including osteolysis, ectopic bone formation, and inflammatory response)2, and prompt the need for new carrier materials for optimizing BMPs spatiotemporal delivery. Our study aimed to evaluate the regeneration of a critical-sized segmental bone defect in a sheep preclinical model, with a 3D architectured poly L-lactic acid (PLLA) scaffold coated with a biomimetic film containing BMP-2. The osteoinductive properties of these films deposited on different types of implantable materials have previously been demonstrated both in vitro3,4 and in vivo.5–7

Methodology
PLLA cylindrical scaffolds (25mm high, 14mm in diameter and with cubic pores of ~880µm) were 3D printed, coated with a biomimetic film previously developed by members of our team8,9 and subsequently loaded with BMP-2. A 25mm-long mid-diaphyseal segmental metatarsal bone defect was created and stabilized with a plate in 9 sheep. Defects were filled with either (i) PLLA scaffold loaded with (from 300 to 600 µg) BMP-2 (n=7) or (ii) BMP-2-free PLLA scaffold (n=2). Monthly radiographic follow-up was performed until animal sacrifice at 4 months. The newly-formed bone between the defect edges and within the scaffold was quantified in explanted specimens with micro-CT. Specimens were then processed for undecalcified histology to characterize bone formation/resorption, bone-scaffold interface, inflammatory response and vascularization of tissue.

Results
Consistent radiographic bone union was observed in 7/7 animals when BMP-2 containing film-coated PLLA scaffolds were implanted, whereas none of the 2 animals implanted with the BMP-2-free PLLA scaffold did achieve bone union. Neither abnormal bone resorption nor chronic inflammatory response were observed with the bioactive scaffold containing BMP-2. Dense newly-formed bone filled with numerous osteocytes was observed all around and in direct contact with the scaffold material pillars.

Conclusions
3D printed PLLA scaffolds coated with a biomimetic films containing BMP-2 provided consistent radiographic bone union in a preclinical critical-size segmental defect without noticeable deleterious effects. This strategy opens new avenues for the replacement of segmental bone defects.

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comment:This work was supported by the National Agency for Research (ANR OBOE-28-CE17-0016). Catherine Picart is grateful to be a senior member of the Institut Universitaire de France whose support is acknowledged

keywords: 3D printed scaffold, Bone morphogenetic protein, bone regeneration, critical-sized defects, pre-clinical sheep model

62825444649

NEW SURFACE FUNCTIONALITIES FROM GRAFTING NATURAL BIOMOLECULES TO TITANIUM ALLOYS

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Introduction
Several molecules of natural origin are of great interest to add specific surface functionalities to implant biomaterials: phenolic compounds and keratin can be used for different targets, as an example. They are derived from industrial processing of respectively plants or animals derivatives through a valorization process of waste and a sustainable circular economy approach. The modified surfaces enhance and fast the tissue integration, fight or reduce the risk of infections, guide the tissue growth, modulate the inflammatory response.
Different processing can be followed: grafting of a molecular monolayer (functionalization), thin or thick continuous coatings. The selection of the processing must be guided by the chemistry of the surface and ligands as well as by the expected mechanism of action of the biomolecule: progressive release or permanent link to the surface. Functionalization or coating can be coupled to different surface topography of the substrate for a synergic chemical-physical effect on the host response.
Post-processing (packaging, sterilization, storage) of the final products must be adapted to the presence of the biomolecules, too.
Potential applications are in orthopedic, dental, cardiovascular implants.

Methodology
Polyphenols (phenolic acids, flavonoids, and condensed tannins) are extracted from organic red grape pomace. The functionalization process is performed at pH = 7.4 with the addition of calcium ions, which act as a bridge between the substrate and polyphenols. The presence, amount (semi-quantitative), distribution, release, and type of bonding to the surface of the grafted polyphenols have been assessed.
Using electro-spinning, mirror-polished Ti disks were uniformly coated with keratin fibers obtained from discarded wool via sulfitolysis; surface functionalization with keratin molecules has been released, too. The keratin modified surfaces were then doped with silver (Ag) to introduce antibacterial properties.
The resulting specimens were characterized in terms of morphology and chemical composition by FESEM, FTIR, zeta potential titration curves, KPFM, and XPS. The antibacterial properties of the Ag-doped specimens were tested against a multidrug-resistant Staphylococcus aureus biofilm through morphology (FESEM) and metabolic assay. Lastly, the cytocompatibility of the specimens was confirmed using human primary gingival fibroblasts and mesenchymal cells.

Results
The functionalized samples have a homogeneous distribution of polyphenols as a continuous layer and micro-sized agglomerates. The grafted polyphenols maintain redox chemical and radical scavenging ability. A fraction of polyphenols is released into water in one day, while a firmly grafted layer remains on the surface even after four weeks. A larger release can occur in

case of an environment with pH of 4–5 (e.g. inflammation). The functionalized surfaces can be sterilized by gamma irradiation without significant damage of the grafted polyphenols. Concerning keratin, the Ag surface enrichment was effective in reducing viability and maturation of S. aureus biofilm, without compromising human cell viability. The cell spread was found to be very sensitive to keratin fiber stimulation.

Conclusions
Both the strategies thus appear to be very promising to introduce surface features in line with the main requirements for transmucosal and bone implants and it is of great interest to compare them in terms of efficacy and target application.

keywords: surface, dental, orthopedic, keratin, polyphenols, functionalization, coating

20941847799

ENGINEERING OF A BRIDGE PROTEIN TO IMPROVE THE DELIVERY OF BMP-2 FROM COLLAGEN SPONGE AND ENHANCE BONE REGENERATION FOR SPINAL FUSION

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Introduction
The bone morphogenetic protein-2 (BMP-2) is one of the most potent growth factors for bone repair. In the clinic, BMP-2 is widely used for spinal fusion, particularly in the product called InFUSE Bone Graft® (Medtronic). Despite its strong efficacy, the safety of BMP-2 remains questionable as some treated patients suffer from serious side-effects, such as ectopic bone formation, nerve damage, severe inflammation and cancer. In this project, we engineered a bridge protein that effectively slowed the release of BMP-2 from collagen sponges, the carrier material used in InFUSE Bone Graft®, thus allowing significant dose reduction of BMP-2 for bone repair.

Methodology
The bridge protein was designed for dual affinity to collagen I and to BMP-2. Specifically, it was made by the fusion of the fragment antigen-binding (Fab) of an anti-collagen antibody to the growth factor-binding domain of laminin, which displays high affinity to BMP-2. The bridge protein was produced in Human Embryonic Kidney (HEK) 293 mammalian cells and purified by affinity-mediated chromatography. In all experiments, the bridge protein was simply admixed to BMP-2 prior to incorporation into collagen sponges. We first tested the efficacy of the bridge protein in slowing BMP-2 release in vitro. Then, we assessed the therapeutic efficacy of BMP-2 ± bridge protein, delivered in collagen sponges, in two in vivo mouse models of bone regeneration, a critical-size calvarial defect model and an intervertebral defect model newly developed by us to mimic spinal fusion in mice. Bone regeneration was monitored via in vivo CT scan imaging.

Results
In vitro, the bridge protein strongly enhanced the retention of BMP-2 into collagen sponges. Indeed, BMP-2 was released over more than 7 days versus about 3 days in presence or absence of the bridge protein respectively. Upon single implantation in vivo, the bridge protein permitted significant improvement of bone regeneration at very low doses of BMP-2, as measured by the volume of newly formed bone, the defect coverage and the rate of spinal fusion. Positive results were consistently observed in both the calvarial and intervertebral defect models in mice [1]. In addition to retention in collagen sponges, we demonstrated that the bridge protein allowed local retention of BMP-2 in the endogenous collagenous extracellular matrix (ECM) of tissues.

Conclusion
We engineered a bridge protein that substantially improved the delivery of BMP-2 from collagen-based materials. Combining the bridge protein to BMP-2 significantly enhanced bone regeneration in vivo, therefore allowing good therapeutic efficacy at very low dose of BMP-2. Such protein engineering approach for growth factor delivery may be generalizable to many other applications of tissue engineering, considering the broad use of collagen-based materials in regenerative medicine (e.g., collagen sponges, collagen hydrogels, decellularized matrices).

1. Briquez, P. S. et al., Sci. Adv. 7:eabh4302 (2021)

keywords: Spinal fusion, BMP-2, Collagen material, Protein engineering, Bone regeneration

83767245227

PCL REINFORCED COLLAGEN SCAFFOLDS FOR ENDOCHONDRAL HEALING OF BONE DEFECTS

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Collagen scaffolds are well known for their regenerative potential. However, the relatively poor mechanical properties represent a problem (scaffold deformation, partial collapse of internal open pore structure) for the translation into a clinical setting. In this study, we present a novel approach: A Mechano-Hybrid-Scaffold (MHS) that combines a collagen-based biomaterial with highly aligned channel-like pores with a 3D printed poly(ε-caprolactone) (PCL) support structure [1], overcoming contradictory requirements for mechanical stiffness and scaffold architecture.

The previously described orientated collagen scaffold [2] was combined with a 3D printed PCL macro-porous support structure [3] that preserves the internal architecture of the collagen scaffold. Internal architecture characterization (scanning electron microscopy imaging) and mechanical characterization (monoaxial compression test) were performed. Crosslinking and degradability were assessed by determination of the denaturation temperature of the collagen (Td).

MHS were successfully produced with stiffness of 9.56 MPa (stiff) and 0.01 MPa (soft) for the supporting structure, respectively. MHS characteristics, e.g. Td (79.8 ± 0.1 °C) and pore size (78.1 ± 18.1 μm), remain the same as the ones of collagen reference scaffolds without a PCL supporting structure. Scanning electron microscopy images show full integration of the support structure inside the collagen structure and no alteration of the scaffold inner architecture.

With this approach, mechanical characteristics can be tuned independently at the micro-scale (cell-level) and the macro-scale (tissue-level). The MHS opens the door for new applications of collagen scaffolds in rigid tissue regeneration by solving the paradox of providing soft cell environment and high structural stability in implantable materials.

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The authors acknowledge financial support by the German Federal Ministry of Education and Research (BMBF) via grants no. 13XP5048A-D).

keywords: collagen, pcl, endochondral

62825451306

MICROSTRUCTURE EFFECT ON BONE FORMATION OF A FUNCTIONALLY GRADED SCAFFOLD USING A MECHANOSTAT-BASED MODEL

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From a mechanical point of view, bone demonstrates exceptional mechanical properties owing to its complex hierarchical composite structure. The human skeleton acts as a support for the whole body as it withstands stresses produced by daily routines and gravitational force. As a result of these stresses, the bone regulates its geometry and density through activating formation and resorption mechanisms (Pivonka, 2018). Different mechano-regulatory theories were developed to address the dependency of the bone remodeling mechanism on mechanical stimulation at multiple scales. At tissue scale, where the bone is considered as a homogeneous material, the strain energy density (SED), effective stress, octahedral shear strain and interstitial fluid flow have been examined as the driving force for bone formation and resorption mechanisms. Advances in computational power and numerical techniques have extended the ability to apply these mathematical schemes to large-scale problems involving the design and optimization of scaffolds for large bone defects. Numerical schemes, such as finite element method (FEM), boundary element method (BEM), and meshless methods have been used to simulate bone remodeling, among which, FEM is by far the most used method (García-Aznar et al., 2021). Design of scaffolds faces numerous challenges at different scales and physics. One of the ongoing challenges is to optimize the microstructure of the scaffold to maximize the efficacy of the scaffold as a supporting structure for bone formation. For this reason, functionally graded scaffolds (FGSs) are designed which closely resemble the mechanical, biological, and morphological properties of the bone structure (Zhang et al., 2018). In this study, an FEM-based approach (Shi et al., 2018) was adopted to investigate the effect of porosity variation on bone formation inside an FGS incorporating both degradation and regeneration. The SED-based feedback mechanism was employed to consider the effect of mechanical stimuli on bone formation of a FGS. Bulk, surface, and stochastic degradations were considered in modeling of the scaffold degradation for the first time for a FGS. The aim of this study is to evaluate the effect of microstructure on the bone formation inside an FG bone scaffold and establish the basis for potential future studies on optimization studies of FGSs for maximum performance. The reported results can be used as benchmark solutions for future numerical analysis of the bone formation inside FGSs and serve as a means to validate future in-vivo or in-vitro experimental results.

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keywords: FEM, lazy zone, mechanobiology, FGS, strain energy density

94238177319

EFFECT OF 3D SCAFFOLD MORPHOLOGY ON BONE TISSUE REGENERATION BASED ON A MULTI-PHYSICS FEM MODEL

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Design and fabrication of optimal scaffolds for bone tissue repair is a multi-physics problem targeting osteoconductive, biodegradable material designs with loading of necessary growth factors. Therefore optimal tissue regeneration based on accurate analysis models including the degradation behavior of biomaterials is a critical design requirement (Byrne et al., 2007). In order to explore bone regeneration, cell response to growth factors and existing intracellular signaling pathways driving bone regeneration through transcription factors should be taken into consideration considering degradation of the scaffold (Sun et al., 2013). In this work, this problem is addressed by solving time dependent hydrolysis reaction equations coupled to a reaction-diffusion equation and a set of ordinary differential equations representing release of growth factors and intracellular signaling pathway, respectively. The evolution of bone scaffold degradation, growth factor release and intracellular signaling pathway are conducted as a parametric study based on the effect of pore size of the scaffold within a FEM based modeling environment. Parameters such as degradation rate constant, diffusivity of water through scaffold are tuned based on existing computational models and experimental data in literature. In addition to providing an efficient tuning ability for design parameters, computational models offer significant advantages such as time and cost savings of experimental design and validation processes (Wang et al., 2020). Therefore, the aim of this study is to analyze the effect of porosity on the scaffold degradation, growth factor release and cell response during the bone healing process for a regular structured 3D cubic scaffold with aligned pores based on coupled reaction-diffusion PDE equations. COMSOL Multiphysics® was used to create a unified modeling framework that should allow for additional multi-physical effects such as mechano-biology based regeneration, diffusion of MSCs and angiogenesis to be considered within future design studies. Degradation profiles for scaffolds with different pore sizes and porosities were analyzed and the effect of degradation on growth factor release profiles and cellular response were observed. It has been shown that the pore size and porosity of a bone scaffold affects the bone regeneration process through dynamic interaction between growth factor release profiles and their regulatory mechanism on transcription factors with scaffold degradation.

keywords: Scaffold degradation, signaling pathway, growth factor release, bone healing

41883649528

DELIVERY OF MESENCHYMAL STROMAL CELLS USING COLLAGEN MEMBRANES EMBEDDED IN LEGO®-INSPIRED MULTICOMPONTENT SCAFFOLDS FOR PERSONALISED MANDIBULAR DEFECT REPAIR

Luan Phelipe Hatt (AO Research Institute, Davos Platz, Switzerland), Sylvie Wirth (AO Research Institute, Davos Platz, Switzerland), Angela Rita Armiento (AO Research Institute, Davos Platz, Switzerland), Aapo Ristaniemi (AO Research Institute, Davos Platz, Switzerland), Keith Thompson (AO Research Institute, Davos Platz, Switzerland), Dominic Gehweiler (AO Research Institute, Davos Platz, Switzerland), David Eglin (Health Engineering Center, Mines Saint-Etienne, France), Martin James Stoddart (AO Research Institute, Davos Platz, Switzerland)

3D-printed personalised scaffolds are an attractive approach for mandibular bone repair. Poor mechanical stability of medical grade ceramics is a disadvantage. Also, delivery and retention of regenerative cells within 3D-printed scaffolds remains a challenge. This work aims to create 3D-printed personalised scaffolds based on a novel combination of materials with enhanced mechanical and cell-adhesion properties and with a configurable layered composition including cell-laden collagen membranes for improved cell delivery. The printable ink is created in ethylene carbonate (EC) and consists of 40% (w/V) PLGA to support printability and mechanical strength, 20% (w/V) β-TCP to increase osteoconductivity and 10% (w/V) TPU for elasticity. Solvent-based printing is applied using the RegenHu 3D Discovery® Bioprinter. A 3D model of a mandibular defect is derived from CT scans, then sliced and modified with CAD to obtain LEGO®-like structures. The personalised scaffolds are printed as a series of layers incorporating an interlocking mechanism. Human bone marrow derived mesenchymal stromal cells (MSCs, obtained will full ethical approval) are seeded and kept for 21 days under osteogenic culture conditions on two commercially available collagen membranes: 1. Lyostypt (B. Braun) and 2. Collagen Cell Carrier (CCC) (Viscofan Bio Engineering) (N=3). Viability of MSCs on the collagen membranes placed in between 3D-printed scaffold layers is examined via live-dead staining after 8 days of culture (N=1). Water-mediated EC removal leads to surface microporosity and roughness, both confirmed by SEM, and both favourable properties for improved cell adhesion. Mechanical compression test (N=10) of the 3D-printed scaffold shows improved stiffness and ductility compared to the commercially available ceramic Osteoink®. Under osteogenic culture conditions, MSCs seeded on collagen membranes have increased alkaline phosphate activity at day 14 compared to controls, with an effect more profound for the CCC. When MSC are seeded on the collagen membranes, expression of bone sialoprotein mRNA is upregulated not only in the osteogenic medium but also in the control medium. Live-dead staining shows good cell survival on the Lyostypt cultured in between 3D-printed scaffold layers, while a higher number of dead cells are detected on the CCC. 3D-printed scaffold biocompatibility and cell proliferation is shown by live-dead staining over 8 days of cell culture. Large scale personalised mandibular implants can be successfully printed, assembled and combined with cell-laden collagen membranes. We propose a novel 3D printable ink for mandibular bone reconstruction as an alternative to ceramics. Ongoing tests aim to demonstrate that osteogenic capabilities of the 3D-printed scaffold and efficient seeding of biologics intraoperatively to promote osteogenesis and vascularisation.

keywords: 3D-Printing, Mandibular, Collagen Membrane, MSCs

94238145577

COMPOSITE BIOMATERIAL-INK WITH HYALURONAN, COLLAGEN AND CALCIUM PHOSPHATE PARTICLES FOR DELIVERY OF CHEMICALLY MODIFIED RNA TO PROMOTE BONE REGENERATION

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Introduction
Most bone defects heal successfully, however, there is an increasing number of cases where bone self-healing is insufficient. Thus, there is a high need for scaffolds able to replace the clinical gold standard treatment autologous bone grafting, which entails donor site morbidity and lacks control over spatial architecture to match defect sites. Biofabrication offers great potential to produce constructs that provide control over shape, architecture and composition. Therefore, this study aims to develop a 3D-printable composite biomaterial-ink to fabricate patient-specific bone graft substitutes for bone regeneration. Based on the heterogenous nature of bone, the biomaterial ink combines inorganic osteoinductive calcium phosphate particles (CaP) with tyramine modified hyaluronic acid-Collagen type I (THA-Col) organic matrix for the delivery of chemically modified RNAs (cmRNAs) inducing nerve, vessel, and bone formation.

Methods
Biomaterial-ink formulations consisting of 17.5 mg/mL THA, 2.5 mg/mL Col with 1 U/mL horseradish peroxidase (HRP), and 0.02% w/v Eosin Y, were combined with a range of 0-30% w/v CaP of size 45-63 or 45-106 μm. 0.17 mM H2O2 was added for enzymatic pre-crosslinking, to create a viscoelastic gel with shear thinning properties. After extrusion of desired structure, further gelation was triggered by light crosslinking for 30 minutes (505 nm). 1% v/v Nanocapsules, as vectors for the cmRNA, were mixed into the pre-polymer solution, gelation and distribution of Nanocapsules within the ink was then analyzed. Composites were further characterized for printability, cohesion, swelling, degradability, and compression modulus. Printability of formulations was evaluated by printing a continuous strut, line spacing, lattice, and overhanging strut on a pillar structure. Further, biomaterial composites were assessed in vitro using a metabolic activity assay after 1, 3 and 7 days using human mesenchymal stem cells (hMSCs).

Results
All formulations were viscoelastic and extrudable, with the formation of a continuous strut, good shape retention and without waviness. The addition of cmRNA vectors resulted in homogeneous dispersion within the matrix and did not influence the gelation mechanisms. All printed formulations retained their original weight and macroscopic shape when lyophilized and rehydrated. Additionally, formulations of THA-Col showed higher metabolic activity compared to THA alone. The range of identified formulations is being assessed for in vitro osteogenesis of hMSCs (viability, mineralization, alkaline phosphatase (ALP) production, gene expression, and protein production).

Conclusion
Here, a 3D-printable composite THA-Col/CaP biomaterial-ink was developed that is suitable for

the combination with cmRNAs/vectors and holds significant potential as bone graft substitute for bone regeneration.

keywords: Bone regeneration, 3D-printing, composite biomaterial-ink

S45
Nature bioinspired biomaterials and strategies for TERM
Room: S3 A
(30 Jun 2022, 15:30 - 17:00)

Conveners: Thomas Groth; Nuno Neves

31412714088

CONDUCTIVE HYDROGEL NANOCOMPOSITE-BASED NEURAL INTERFACE FOR IN VIVO RECORDING OF BRAIN CORTEX SIGNALS

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Introduction
The acquisition of neural signals from the brain cortex has always been of high relevance in the field of neuroscience in order to analyze and interpret brain processes as well as individuate neurological diseases and disorders [1]. Nowadays, the design of a neural interface conformable with the brain tissue is one of the major challenges, since the inadequate conformability might lead to inaccurate signal recording and potential misdiagnosis [2].

Methodology
In this research, we design and produce a soft neural interface composed of polyacrylamide hydrogel loaded with plasmonic silver nanocubes to provide the system with good electrical properties. The hydrogel nanocomposites are surrounded by a template of two layers of silicon-based materials (i.e. polydimethylsiloxane and soft skin adhesive) as supporting elements for guaranteeing a tight and stable neural-hydrogel contact, while allowing a stable recording from specific locations of the brain cortex.

Results
The morphological, chemical, electrical, and mechanical properties of the platform are evaluated. The hydrogel nanocomposites show superior conductivity properties, while mimicking the brain tissue mechanical characteristics. Furthermore, in vitro biological tests performed by seeding neural progenitor cells reveal the biocompatibility of the hydrogel-based system as well as neural differentiation and proliferation. In vivo experiments on a mouse model demonstrate that the hydrogel nanocomposite-based neural interface permits the efficient recording of neural signals with augmented amplitude. Additionally, chronic neuroinflammation tests reveal no adverse response towards the proposed platform.

Conclusions
The biocompatible conductive hydrogel nanocomposite-based device is a promising candidate as neural interface for brain signal acquisition without provoking neuroinflammation. The potential exploitation of the proposed conductive hydrogel platform in electronic devices for Electrophysiological Recording of Electrocorticographic or Electroencephalography Recording will be investigated in the near future.

Acknowledgments
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keywords: nanocomposites, conductive hydrogels, neural recording

62825415955

BIO-ENGINEERING OF A XENOGENEIC VASCULARIZED ENDOCRINE PANCREAS (VEP) FOR TYPE 1 DIABETES

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Background: Intrahepatic islet transplantation in patients with T1D is limited by donor availability and lack of engraftment. To overcome these limitations, based on our experience with decellularized rat lung as scaffold for the generation of Vascularized Islet Organ (VIO, lung scaffold repopulated by murine islets and HUVEC cells), we engineered an upgrade based on human blood-derived endothelial cells (BOECs - Blood Outgrowing Endothelial Cells) and immature neonatal porcine islet clusters (NPIs)

Methods: NPIs and BOECs phenotype profile was assessed by flow cytometry, insulin secretion test and tube formation assay. Rat lung was decellularized with SDS and Triton and seeded with NPIs and BOECs, generating a Vascularized Endocrine Pancreas (VEP). VEP was cultured for 7 days in a customized bioreactor specifically designed to allow cell integration. The β cell death in mature VEPs was estimated during ex vivo organ maturation evaluating miR-375 expression ddPCR compared to batch matched NPIs in standard condition. Matured VEPs and control NPIs function were measured by dynamic glucose perifusion and insulin quantification (by ELISA/IF). Thus, VEPs were subcutaneously transplanted in diabetic immunodeficient NSG recipients and compared with matched NPIs transplanted in different implantation sites: kidney capsule (KC-NPIs), deviceless (DL-NPIs) and liver (LV-NPIs).

Results: Matured VEPs showed a regenerated vascular network (CD31+) with NPIs (insulin+) integrated. miR-375 was expressed in NPIs but not in BOECs, as expected. VEP was able to significantly reduce β cell death ($p<0.05$). Matured VEPs were able to sustain NPIs engraftment, survival and significantly improve insulin secretion during the maturation process compared to batch matched NPIs cultured in standard conditions (AUC VEPs first phase: 3.765±0.90; NIPs 1.60±0.25 $p<0.01$). In long-term transplants in diabetic mice, VEPs demonstrated a significant NPIs engraftment with a prompt function after implantation and the reversal of the glycaemia within 2 days until 60 days after implantation, showing significant superior function compared to all the internal controls (KC-NPIs, DL-and LV-NPIs).

Conclusions: VEP technology is able not only to foster the NPIs functional endocrine maturation in vitro but also to immediately perform in vivo upon transplantation for over 2 months, compared to normal performance within 8 weeks after implantation in different state of the art preclinical models. Given recent progress in genetic engineering of NPI donor pigs, this technology may enable assembly of immune-protected functional personalized endocrine organs. VEP is the first organ to our knowledge assembled with relevant source of endocrine and endothelial cells suitable for future clinical translation.

keywords: Type 1 Diabetes, organ engineering, cell therapy, islet transplantation, xenotransplantation

94238144919

4D BIOPRINTING OF A DYNAMIC MULTI-MATERIAL SCAFFOLD FOR IN VITRO MODELING OF NEURAL TUBE DEVELOPMENT

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Four-dimensional (4D) bioprinting (i.e., fabrication via additive manufacturing of scaffolds characterized by a programmed change, over time, under a predefined stimulus [1]) can be exploited to produce active scaffolds that can modify their shapes upon desired stimulation, thus potentially recapitulating biological processes such as morphogenesis.
In this study, we exploited the 4D bioprinting approach to design and fabricate an innovative smart scaffold for in vitro modeling the development of the neural tube (NT), the structure from which the central nervous system stems in the embryo, with the final aim to guide stem cells towards neural differentiation. The smart scaffold is able to self-fold in time, mimicking the neural plate folding to create a hollow tube, namely the NT [2].
The requested behavior is achieved exploiting the differential swelling properties of bilayer films [3]. Indeed, the different volumetric swelling of the two layers, when dipped in water, creates a deformation mismatch in the film that leads the folding of the film itself. In this study, the two layers were made of the same bulk material (i.e., gelatin crosslinked using (3-Glycidoxypropyl) methyldiethoxysilane, GPTMS-GEL), thus guaranteeing a chemical bond between the layers and avoiding delamination. The swelling behavior of the layers was tuned through the modification of the GPTMS and GEL concentrations.
GPTMS-GEL-1 monolayer films, with the higher volumetric swelling, were fabricated by solvent casting. Then, lines of GPTMS-GEL-2, with lower volumetric swelling, were deposited on the GPTMS-GEL-1 film by Extrusion-Based Bioprinting. The presence of precisely oriented lines (as second layer of the bilayer film) provides a constrain and, as a consequence, a complete control over the film folding direction.
When dipped in water the film self-folds, maintaining its shape in time and, as expected, the orientation of the folding depends on the printed line direction.
Cellular tests have been performed to verify the properties of the smart scaffold, using human induced pluripotent stem cells (iPSCs) directed toward neural progenitor fate via Dual SMAD inhibition. iPSC-derived neural progenitor population uniquely recapitulates in vitro the onset of the founder population of the developing NT. Indeed, the simultaneous application of these cellular and bio-engineering technologies will provide a platform to assess complex phenomena such as NT folding and cellular polarization in a dynamic 4D environment. This pioneering platform will provide an innovative standpoint to unravel neural tube defects and their clinical impact.

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keywords: 4D bioprinting, neural tube, in vitro model, neural differentiation

83767241505

ELECTROSPINNING AND METAL STENTS – A GOOD FIT?

Konstantina Kanari (The Electrospinning Company Ltd, Didcot, Poland)

Micro- and nanofibrous materials have attracted the interest of the scientific community for the better part of ~30 years, due to their unique properties and potential for applications. Electrospinning is a technique that can produce micro- and nanofibers fast, repeatedly, and with tailored morphology. When fibres are electrospun from biocompatible polymers then they can be used in numerous medical applications such as tissue engineering, regenerative medicine, implant coatings, medical textiles, etc. This academic focus has slowly translated into a number of medical devices containing electrospun biomaterials becoming clinically available. The particular area of interest for this presentation is the use, substitution, and improvement that electrospun textiles offer in the field of medical textiles as metal stent coatings.

A brief overview of the state-of-the-art of woven textiles as well as in use in metal stents will be given, as well as a summary of existing electrospun metal stent coverings available now or in clinical trials.

At The Electrospinning Company Ltd we design, develop and manufacture advanced micro- and nanofibrous materials. These biocompatible materials can be used in a range of different biomedical applications, including wound care, cell therapy, and cardiovascular devices. Currently our materials are being used in at least four FDA-approved medical devices that are in the clinical trial stage.

Together with our partners we are developing different types of electrospun textiles for various metal stent applications. We will present data on direct comparisons between traditional textiles and electrospun textiles and their performance in different metal stent applications, such as EVAR, TEVAR, occluders, and covered vascular stents. Examples of the parameters discussed are diffusion across the coverings, blocking of particles, as well as adhesion to the metal frame.

As a centre of excellence in electrospinning biocompatible materials, we have an in-depth understanding on the relationship between material characteristics, like fibre morphology and architecture, and their suitability for specific applications. Therefore, it is paramount to ensure that these characteristics remain stable while the adhesion of the fibres on the different substrates is enhanced.

keywords: electrospinning, biomaterials, fibres

52354566648

FROM PROTEIN-BASED LIQUIFIED MICROCAPSULES TO BONE TISSUE MICRO-UNITS

Ana R. Pinho (CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal), Dora C. S. Costa (CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal), Maria C. Gomes (CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal), João F. Mano (CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal)

Bone tissue regeneration (BTR) has been trying to mimic the bone environment in biofabricated platforms. Given the complex bone metabolism, creating a functional, differentiated and biologically compatible platform that stimulates tissue formation in an autonomous way is currently a challenge [1]. Therefore, the development of multifunctional micro-platforms easy to produce and to translate into the clinic while promoting BTR is a top priority. In this work, we boosted a recently developed platform fabricated in combination with metal coordination and gelling properties of gelatin [2], providing a suitable microenvironment for applications in BTR.

Using gelatin modified with two different catechol analogues (Hydroxypiridinone-HOPO, and Dopamine-DA) as building blocks on the creation of liquefied protein-based microcapsules (mCap), we encapsulated bone-marrow human mesenchymal stem cells (BM-hMSC) using the electrospray technique. The presence of HOPO allowed the formation of the micro-hydrogel shell through metal coordination, while DA was inserted by its affinity for calcium ions allowing the mineralization of the system by deposition of calcium-phosphate crystals (e.g. hydroxyapatite-HA) [3].

With this strategy, we created the desired microenvironment for mineralization and osteogenesis without the demand of osteogenic inducers. With BM-hMSCs organizing themselves inside the inner wall of the gelatin shell, mCap created an encouraging environment for cell communication and differentiation. The inclusion of DA into the system prompted accelerates the differentiation process, with osteoblastic stages being reached in early periods of culture. The prompt differentiation into osteoblasts might be related to the bioactive properties of the mCap, autonomously promoting the deposition of HA crystal. A more detailed analysis revealed the formation of a dense mantle in the interior of this enclosed system, exposing matrix deposition covering osteoblasts. The fact that osteoblast can merge within the new bone matrix suggests that this micro-platform can potentiate the formation of osteocytes, supporting this as a suitable biomimetic platform to closely resemble bone morphology. Therefore, the achieved bone-like microcapsules are a promising bioengineering platform that induce autonomously and in a fast way osteogenesis. By recreating part of the bone cellular microenvironment and architecture, this platform can be explored to bioengineering more closely the bone niche, addressing a variety of bone defects.

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keywords: Liquified-microcapsules; Catechol analogues; Self-healing; Autonomous Mineralization; Osteogenesis

83767269288

CURVATURE-DRIVEN CELL SUTURING CONTROLS CELL ORGANIZATION AND TISSUE FORMATION INSIDE POROUS BIOMATERIALS

Aaron Herrera (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany), Georg N Duda (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany), Isabel Orellano (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany), Erik Brauer (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany), Alicia Serrano (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany), Ansgar Petersen (Julius Wolff Institute - Charité Universitätsmedizin, Berlin, Germany)

Introduction
Tissue growth in defects is controlled by the local curvature of the substrate and is traditionally regarded to follow the same process of cell organization from large to small defects [1]. At defect dimensions of 100s of μms to a few mms, tissue growth typically follows a centripetal process driven by an inner ring composed of contractile and proliferative cells. In contrast, the closure of very small defects with a diameter of 10s of μms, cell protrusions seem to contribute to defect closure. Here, we investigated how curvature drives cells from the previously described layer-by-layer tissue growth into defect closure and we reveal a curvature-driven “cell suturing” process that is most pronounced in stromal cells.

Methodology
The response of different cell types (i.e., fibroblasts, mesenchymal stromal cells, osteoblasts, pre-osteoblasts and endothelial cells) to surface curvature was characterized using micro-engineered cell culture substrates featuring half-cylindrical environments with controlled curvature variation. Collective cellular self-organization and gap closure was analyzed inside well-defined 3D cylindrical pores as well as in a biomaterial environment featuring channel-like pores with more stochastic geometrical conditions. Lastly, micro-wounds were introduced in an in vitro tissue growth setup to observe the transition between different modes of cell organization during wound healing.

Results
Cellular response to curvature is dependent on the cell type and degree of curvature. While on large curvatures (i.e., diameter > 300 μm) stromal cells prefer to align following the direction of higher curvature, as the curvature increased (i.e., diameter < 300 μm), cells adapt to curvature via two well-differentiated mechanisms: alignment towards the minimum curvature or lifting from the substrate along the maximum curvature. The occurrence of cell lifting was correlated with the distribution of the focal adhesions around the cell and can be regulated by the cytoskeletal stress state or the stiffness of the substrate. Cells without lifting capability lead to a process of centripetal gap closure. However, cellular processes involving cell lifting induced a significantly faster suture-like wound healing mechanism compared to the previously described centripetal gap closure.

Conclusion
Fundamental differences were found in how distinct cell types respond to curvature within gaps of few 100s of micrometers. In living tissues, such gaps could represent meso-scale defects occurring i.a., after tissue delamination consequence of mechanical overloading or injury, but such micro-defects may also be engineered in synthetic porous materials. Based on our findings, cells may be classified into types capable to close micro-defects through an extremely efficient

suture-like process and cell types that favor a layer-by-layer gap filling associated with a circular lumen shape and slower defect closure. Addressing the fast gap closure resulting from the cell suturing-mode in biomaterial strategies is regarded advantageous for material-driven tissue healing and regeneration. This can be achieved by implementing appropriate curvatures into porous materials that provoke cell suturing for the individual cell type of interest [2].

References

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keywords: Tissue regeneration, biomaterials, mechanosensing, wound healing, curvature

20941831055

ENGINEERING FUNCTIONAL MICROVASCULARIZED SKELETAL MUSCLE TISSUE EQUIVALENTS VIA MICROFLUIDIC-ASSISTED 3D WET-SPINNING AND MICROVASCULAR SEEDS

Dario Presutti (Institute of Physical Chemistry - Polish Academy of Sciences, Warsaw, Poland), Nehar Celikkin (Institute of Physical Chemistry - Polish Academy of Sciences, Warsaw, Poland), Francesco Nalin (Institute of Physical Chemistry - Polish Academy of Sciences, Warsaw, Poland), Marco Costantini (Institute of Physical Chemistry - Polish Academy of Sciences, Warsaw, Poland)

Introduction
One of the most challenging and daunting task of tissue engineering is the assembly and integration of functional microvasculature systems within the biofabricated tissue equivalents. Having functional vasculature is of the utmost importance to promote the rapid integration of the host's microvasculature with the engineered one present in the graft, thus enabling an efficient transport of nutrients/removal of wastes to/from the graft. To date, a plethora of strategies have been explored to build such vasculature networks and, in the last decade great progresses have been achieved. In particular, it has been demonstrated that one can generate a vascular network via vasculogenesis – the de novo assembly of endothelial progenitor cells into capillaries – using microfabrication approaches such as microfluidic technology, 3D co-culture models (spheroids and organoids), and biofabrication via 3D bioprinting strategies. However, these milestones are still unsatisfactory and, to date, efficient protocols for the manufacturing of vascularized artificial tissues are still missing.

Methodology
To integrate a functional microvasculature within skeletal muscle tissue, we have developed a microfluidic-assisted 3D co-axial wet-spinning strategy. This technique allows to biofabricate core-shell fibres that were deposited on a rotating drum. Such fibers were composed of alginate (shell) and fibrinogen (core, bioink). Within the bioink, we loaded skeletal muscle precursors (C2C12) and gelatin methacrylate (GelMA) microbeads coated with endothelial cells (EC-GelMA) that acted as endothelium micro-seed units. Such beads were massively produced using a millipede step-emulsification microfluidic device.

Results
Applying the correct bioinks formulations, viable vascularized constructs were obtained, and vasculature micro-seeds supported the formation of a capillary-like network Interestingly, we have noticed that by increasing the core stiffness in the constructs, the endothelial cells have been able to generate vessels with different calibers switching from larger (150 microns) to smaller (50 microns) capillaries.

Conclusions
Our results have shown that: i) 3D co-axial wet-spinning is a valuable strategy to biofabricate and integrate in vitro micro-vessels within a secondary tissue; ii) the stiffness of the matrix microenvironment plays a key role over the fate of endothelial cells influencing the size/number – i.e. architecture - of the resulting microvascular network; iii) Micro-vascular seeds are an interesting solutions for engineering microvascular network, easy to manufacture and use in combination (e.g. by simply resuspension in a bioink) with other biofabrication strategies.

keywords: Vasculogenesis, Tissue engineering, 3D co-axial wet-spinning, Micro-vascular seeds.

S46
New developments of regenerative and tissue modeling products
Room: S1
(1 Jul 2022, 11:00 - 12:30)

Conveners: Xanthippi Chatzistavrou; Faleh Marino

20967806797

VAT-POLYMERIZATION BIOPRINTING FOR TISSUE FABRICATION

Yu Shrike Zhang (Harvard Medical School, Cambridge, United States)

Three-dimensional (3D) bioprinting has emerged as a class of promising techniques in biomedical research for a wide range of related applications. Specifically, vat-polymerization techniques such as digital light processing (DLP), are highly effective methods of bioprinting, which can be used to produce high-resolution and architecturally sophisticated structures. Nevertheless, conventional DLP bioprinting systems are hampered by several key limitations such as their bulky footprints, their insufficient multi-material bioprinting capacities, and their usual requirements on mechanically strong materials for volumetric bioprinting due to the layer-by-layer fabrication mechanism. In this talk, I will discuss our recent efforts on developing various DLP-based platforms that successfully tackle these challenges. These platforms will likely provide new opportunities in constructing functional regenerative and tissue modeling products in the future.

keywords: bioprinting, biofabrication, bioink, digital light processing, tissue

LEVERAGING ADVANCES IN BIOMATERIALS AND TISSUE ENGINEERING FOR REPARATIVE, REGENERATIVE AND TISSUE MODELLING SOLUTIONS

Nureddin Ashammakhi (Michigan State University, United States)

Leveraging advances in biomaterials and tissue engineering, it is becoming possible to develop successful reparative, regenerative and tissue modelling solutions. Over more than two decades, we have made progress with studying biodegradable materials for reparative medicine. For example, biodegradable screws and plates were translated to the clinic as osteosynthesis implants. Scaffolds for in situ tissue engineering were investigated and applied for guided tissue regeneration, some of which were also translated to the clinic. We have also developed osteoconductive scaffolds for cell-based tissue engineering. To make scaffolds biomimetic, we developed nanofiber-based scaffolds made of different polymers and optimized their processing techniques using electrospinning. Because different biodegradable polymers elicit inflammatory reaction during their degradation process, controlled tissue response properties were added by the inclusion of anti-inflammatory drugs in these scaffolds. To improve the two-dimensional structure of the scaffolds, three-dimensional (3D) composite constructs were developed and their effect on preserving cell phenotype was demonstrated. To better control cell distribution in scaffolds, 3D bioprinting was pursued for developing cell-laden constructs. To improve cell survival in engineered scaffolds novel bioink based on improved nanostructure was developed and effect on improved cell survival was sown. In addition, the development of oxygenated bioink was pursued and its effect on enhanced cell survival was achieved. A new osteopromotive cell-survival enhancing bioink was developed. In addition to advances, dynamic flow is needed to better mimic native tissue environment. Integration of microfluidic systems and other construct processing techniques is needed to be able to produce advanced biomimetic tissue constructs useful for clinical applications as regenerative tools or as tissue models for disease studies and drug development. To achieve this, multidisciplinary approach and sustained funding are required.

94238112159

A VOCAL WORKOUT: NOVEL BIOREACTOR FOR THE IN VITRO CULTURE OF VOCAL FOLD REPLACEMENT TISSUES

Anja E. Luengen (Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany), Rodrigo Salazar Ortiz (Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany), Christian Boehm (Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany), Martin Kiel (Faculty of Electrical Engineering, University of Applied Sciences and Arts Dortmund, Dortmund, Germany), Stefan Jockenhoevel (Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany, Aachen-Maastricht Institute for Biobased Materials (AMIBM), Maastricht University, The Netherlands), Anja Lena Thiebes (Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany), Christian G. Cornelissen (Clinic for Pneumology and Internal Intensive Care Medicine (Medical Clinic V), RWTH Aachen University Hospital, Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University Aachen, Germany)

Introduction
Laryngeal cancer is often diagnosed at an advanced stage when treatment options are limited and most often restricted to laryngectomy. As this procedure requires a permanent tracheostoma, it is associated with additional complications such as a high risk of infection, fistulae formation and loss of the natural voice. In addition, restoration of the most important laryngeal function, protection of the airways, usually leads to deterioration of other functions like speaking or swallowing. To date, no method exists to simultaneously restore all laryngeal functions, resulting in a poor quality of life in the long term.
In future, a tissue engineered autologous laryngeal replacement could provide a way to ensure airway protection and voice production in parallel. A tissue engineered vocal fold would represent a milestone in developing a complete laryngeal replacement. As the native vocal folds undergo a broad variety of mechanical stresses in vivo such as tension, shear and impact, they exhibit specific biomechanical characteristics that also have to be met by tissue engineered constructs. In this study, we therefore developed a bioreactor that combines vibrational stimulation and stretching for the in vitro culture of vocal fold replacement tissues.

Methodology
To fulfil the tissue’s in vivo properties, we targeted a bioreactor with a frequency range between 100 and 300 Hz in combination with 20 % tensile strain, allowing alternating stimulation patterns with resting periods. Compatible scaffolds for cell seeding include elastic membranes and hydrogels. In addition, we aimed for a reusable, sterilizable and straight-forward design that is easy to implement. Electrical components were designed to avoid contact with the humidified incubator atmosphere, thus enabling long-term cultivation over several days to weeks.

Results
We developed a vocal fold bioreactor consisting of a transparent polymethyl methacrylate (PMMA) cylinder, polyoxymethylene (POM) scaffold holders and POM based housing parts. A linear module connected to a stepper motor implements the stretching of the scaffold while piezoelectric patches transmit vibration. Appropriate oxygenation of the medium is achieved via

a silicon tubing loop. Cell compatibility of the bioreactor was evaluated for culture periods of up to 7 days.

Conclusion
Our bioreactor offers new perspectives for in vitro studies on mechanobiological processes in regenerating tissues. In addition, it represents a first step towards developing a vocal fold replacement tissue that may in the future provide new treatment options for laryngeal cancer patients after total laryngectomy.

keywords: vocal fold replacement, mechanical stimulation, vibration, bioreactor design

52354511697

CAN ORAL MUCOSA BE USED IN PRIMARY HYPOSPADIAS SURGERY IN PREPUBERTAL BOYS?

Petra De Graaf (UMC Utrecht, RMCU and Department of Urology, Utrecht, Netherlands), Eline Kuijt (UMC Utrecht, RMCU, Utrecht, Netherlands), Marjon Viester (UMC Utrecht, Department of Urology, Utrecht, Netherlands), George Tsachouridis (UMC Utrecht, Department of Urology, Utrecht, Netherlands), Laetitia De Kort (UMC Utrecht, Department of Urology, Utrecht, Netherlands)

Background
Hypospadias is a common congenital abnormality with varying severity. There may be lack of local tissue during hypospadias surgery. Oral mucosa (OM) is widely used for urethroplasties and repair after failed hypospadias in adults. Little is known about the applicability of OM in prepubertal boys because of eventual hormonal effect of puberty on OM. We investigated the androgen receptor (AR) localization and testosterone sensitivity in OM.

Materials & Methods
Small waste OM fragments of adult patients undergoing urethral surgery were collected under local biobank protocol. AR staining was performed on tissue sections and tissue was used for isolation of oral keratinocytes. Cells were serum starved, exposed to testosterone for different time points followed by immunostaining for AR.

Results
OM of patients with normal testosterone levels showed nuclear AR localization in the basal layer, and cytoplasmic AR localization in the apical layer, whereas OM of an adult hypospadias patient showed low cytoplasmic AR expression. The basal layer was Ki-67 positive, associated with cell proliferation. Oral keratinocytes of adult hypospadias patient exposed to testosterone showed switch from cytoplasmic to nuclear localized AR after 60 minutes, indicating that testosterone signaling was activated.

Conclusions
Oral keratinocytes are sensitive to testosterone. These findings suggest that OM is responsive to testosterone. Therefore an autograft transplant of OM in prepubertal boys might be suitable and able to keep up with the testosterone induced penile growth. In addition, this is an indication that OM cells can be used in tissue engineering for urethral reconstruction in pediatric patients.

keywords: urology, translational science, hypospadias

20941810604

BIOENGINEERING A NOVEL UV-INDUCED SKIN MODEL TO MIMIC THE EFFECT OF ENVIRONMENTAL STRESSORS EXPOSURE ON SKIN HEALTH

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Skin ageing is a multifactorial process attributed to both intrinsic and extrinsic factors. Intrinsic ageing is associated with changes that occur with age such as cumulative molecular and cellular damage, while extrinsic ageing exacerbates these changes through the UV exposure. Nowadays, ageing has become an important issue in the world: life expectancy and population have increased, while social and psychological factors have motivated a stronger desire for healthy ageing and a youthful appearance. Alongside other environmental factors, solar UV radiation is associated with extrinsic skin ageing, as it drives up to 80% of premature skin aging1 and it is one of the most potent carcinogens known. UV radiation induces structural and functional changes in both the epidermal and dermal compartments, such as erythema sunburn, tanning, DNA damage, inflammation, remodelling of the extracellular matrix, changes in epidermal barrier function, cellular proliferation and differentiation. The extent of these effects are mainly due to the degree of constitutive pigmentation of the skin.

Human neonatal skin cells were used to generate in vitro human skin equivalents as previously described in Roger et al.2. A UV irradiator system was used to simulate UV exposure on skin equivalents and they were characterised using a colorimeter, histology, immunofluorescence, melanin quantification and advanced microscopy.

UV-irradiated non-pigmented skin equivalents, which lack melanin protection, reveal structural epidermal changes, sunburn cells, DNA damage in form of cyclobutane pyrimidine dimers, apoptotic cells, and decreased expression of the differentiation marker filaggrin compared to the sham-irradiated non-pigmented skin equivalents. This effect appears to be dose-dependent, with a greater response identifiable following chronic irradiation. Conversely, UV-irradiated pigmented skin tans following chronic exposure, which is confirmed by an increased melanin content and demonstrates the protective effects of melanin by demonstrating a well-differentiated and organised epidermis and an absence of UV-induced damage. This melanin photoprotection is related to the tone of the pigmented skin equivalent, where the lightest skin tone has a greater UV impact than darker skin types.

We describe the characterisation of UV-induced skin equivalents, which recapitulate UV exposure of human skin and the role of melanin, providing a platform to test new and current formulations for cosmetic products designed to protect and treat the skin from harmful UV-exposure and ageing.

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keywords: skin equivalent, photoprotection, pigmentation, ultraviolet radiation

20941856968

NEW HYBRID HYDROGELS FOR APPLICATIONS AS BIOINKS IN 3D PRINTING IMPLANTS

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Introduction
Three-dimensional bioprinting (3DBP) relies on extrusion-based methods for the printing process, however such methods are known to reduce cell viability due to the introduction of shear forces during extrusion. Additionally, the incorporation of solid elements into bioinks such as ceramic particles that may support bone growth, leads to increased shear forces during 3DBP leading to additional decreases in cell viability [1]. An innovative approach to overcoming these limitations is to develop hybrid bioinks where the inorganic osteopromotive components will be chemically linked with the organic matrix. To achieve the greatest degree of homogenization between the osteopromotive component(s) and the polymer matrix, it is required an in situ synthesis method. This work aims to utilize an innovative approach that combines a methacryloyl functionalized collagen-derived gelatin (GelMA) with an Ag-doped bioactive glass (GAB) to deliver a novel osteopromotive and antibacterial hybrid hydrogel material. Chemical, structural, and antibacterial characteristics of the new hybrid material GAB were studied.

Methodology
The synthesis of GelMA is performed as described in the literature [2,3] and then the solution was frozen and lyophilized before storage. For the synthesis of GAB hybrid material, the lyophilized GelMA is dissolved in DMSO, a coupling agent 3-Glycidyloxypropyl trimethoxysilane (GPTMS) is added to the solution. The sol-gel process was used to synthesize the Ag-doped bioactive glass (Ag-BaG) following previously described methods [4]. The combination of the solutions leads to precipitation, then washes and lyophilization to prevent the materials characteristics from changing during storage. Prior to the further use, lyophilized GAB was dissolved in phosphate buffered saline (PBS) along with the photoinitiator and photopolymerized producing GAB hybrid hydrogels. Structural characterization, performance, printability, and antibacterial properties were studied.

Results
Rheological evaluation found the GAB exhibited shear thinning behavior, which is a preferential characteristic for printability. The incorporation of the Ag-BaG was found to be homogenous at the molecular level that led the GAB to exhibit less amount of swelling and the slow degradation behavior compared to GelMA alone. Significant antibacterial inhibition was achieved by GAB against MRSA.

Conclusions
GAB is expected to be suitable for extrusion-based 3DBP technologies and expected to improve cell viability of the 3D printed constructs due to the absence of particulate components. The antibacterial characteristics can advance the performance and success of the printed constructs.

keywords: Hybrid hydrogel, GelMA, Ag-doped Bioactive Glass, Bioink, Antibacterial

20941856968

CLAY BASED STRUCTURED GELS FOR CONTROLLED DELIVERY OF VASCULAR ENDOTHELIAL GROWTH FACTOR

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INTRODUCTION
Vascular endothelial growth factor (VEGF) is the principal regulator of angiogenesis. Tightly bound gradients to extracellular matrix determine the microvascular endothelial cells fate during organogenesis and tissue regeneration and uncontrolled expression can lead to abnormal vascular growth and vascular tumours 1. Despite advances in tissue engineering, tight spatio-temporal control of VEGF remains a challenge hindering its therapeutic application. Nanoclay-gels have established potential in tissue engineering due to their capacity to sequester proteins for sustained and localised bioactivity 2, 3. The current study reports a biomimetic method to applying self-assembling nanoclay-gels comprising 3D gradients of VEGF to support localised spatio-temporal formation of microvasculature.

METHODOLOGY
Hydrous suspension of Laponite®, a synthetic smectite clay, were added to a solution containing biomolecules to facilitate the structured gels assembly via reaction-diffusion. The assembled structures were loaded with punctured VEGF gradients. Its spatial distribution and concentration were confirmed with fluorescent imaging and ELISA, respectively. The biocompatibility and bioactivity were assessed with a human umbilical vein endothelial (HUVEC) tubule formation assay and a 28-day murine subcutaneous implantation. A contrast agent was injected to visualise the new blood vessel formation via µCT and corroborated with immuno-staining.

RESULTS
Structured gels were able to controllably pattern the distribution of VEGF in 3D with a resolution of 40 - 120µm depending on assembly conditions. Patterned gels supported tubule formation of HUVECS grown on gel surfaces and µCT analysis of the in vivo study indicated vascularization of gels within regions of VEGF patterning. This was confirmed by histological analysis showing progressive cell invasion and degradation of the gel, and microvessel formation within the projected area of the VEGF pattern.

CONCLUSIONS
Clay-based structured gels are a promising delivery system of growth factors for tissue engineering applications with clinical relevance. Here we demonstrated for the first time its capacity to hold VEGF gradients for localized bioactivity.

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keywords: Hybrid hydrogel, GelMA, Ag-doped Bioactive Glass, Bioink, Antibacterial

S47
New insights underlying mesenchymal stem cell-mediated bone regeneration
Room: S2
(29 Jun 2022, 15:30 - 17:00)

Conveners: Kamal Mustafa; Cecilie Gjerd

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STEM CELLS IN BONE REGENERATION, A RANDOMIZED CLINICAL TRIAL

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Introduction
In a non-controlled recent clinical study, autologous bone marrow derived stem cells combined with biomaterial induced new bone formation. This has been reported as promising new approach for reconstruction of atrophied posterior alveolar mandibular ridges. We aimed in the present study to demonstrate the efficacy of this therapeutic ATMP approach in a randomized multicenter controlled clinical trial.

Our research group has pioneered the field of bone tissue engineering and demonstrated feasibility, safety, and efficacy of the combination of Biphasic Calcium Phosphate (BCP) granules and autologous bone marrow derived stem cells (MSC) in preclinical studies and early phase human clinical trial (n= 11 patients). Successful regeneration of the alveolar bone in the pilot trial was evident in radiographic and histological findings [1]. The findings were supported by the ability of the newly formed tissue to accommodate a dental implant and withstand the forces of mastication on daily bases. Therefore, the present work is aimed to perform phase II multicenter randomized controlled clinical trial for regeneration of mandibular bones of patients prior to dental implants using autologous MSC.

Methodology
Patients with a need for bone reconstruction of residual edentulous ridges in both the mandible and maxilla due to bone defects with a vertical loss of alveolar bone volume and/or knife edge ridges (≤ than 4,5 mm) unable to provide adequate primary stabilization for dental implants were included in the clinical study. Autologous bone marrow MSC were expanded, loaded on BCP (MBCP+™; Biomatlante, France) and used to augment the alveolar ridges. After five months bone biopsies were harvested at the implant position site and implants were installed in the regenerated bone. The implants were loaded after 8 -12 weeks. Safety, efficacy, quality of life and success/survival were assessed. Five clinical centers, 4 different countries participated. Bone grafts harvested from the ramus of the mandibles were used as control in the study.

Results
41 patients have so far been screened and enrolled in the study. 21 patients have been treated in the test group, 9 in the control group, 6 are waiting for treatment, and 5 withdraw before treatment.

Conclusions

The results this far indicates that the use of bone marrow derived stem cells in the applied protocol for augmentation of the atrophied mandibular ridge have results comparable to the gold standard; autologous bone transplantation with predictable longtime results.

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keywords: Stem cells, clinical trial, bone regeneration

94238140506

BONE-MARROW MESENCHYMAL STEM/STROMAL CELLS HAVE ENHANCED VASCULOGENIC POTENCY OVER ADIPOSE STEM/STROMAL CELLS IN PERFUSED IN VITRO CULTURES

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Insufficient vascularization is a major obstacle for clinical application of tissue engineered transplants including bone. The ambition is to provide an environment rich in vascular networks to achieve efficient osseointegration and accelerate functional restoration after implantation. Of particular interest is the microvasculature that is crucial for oxygen and nutrient delivery. Microvascular networks in 3D can be formed in vitro through the co-culture of endothelial cells (ECs) with supporting pericytic cells. Mesenchymal stem/stromal cells (MSCs) derived from bone marrow (BMSCs) and adipose tissue (ASCs) are an attractive choice for pericytes due to their natural perivascular localization and ability to support formation of mature and stable microvessels. Furthermore, they are most used cell types for bone tissue engineering and clinical trials focusing on bone regeneration.
Here, our aim was to explore the vasculogenic potential of human ASCs and BMSCs in a perfusable microfluidic device.

BMSCs and ASCs were co-cultured with ECs in a fibrin hydrogel in a microfluidic chip. We compared the capacity of BMSCs and ASCs to induce the formation of mature microvascular networks by ECs and to differentiate into pericytes. We studied the effect of MSCs on vessel characteristics such as area, diameter, length, and perfusability. Interstitial flow across the hydrogel area was measured daily in EC-BMSC and EC-ASC cocultures using fluorescence imaging. We assessed MSCs pericytic differentiation in terms of pericyte area and pericyte coverage by immunohistochemical staining and quantitative analysis. Furthermore, we evaluated the expression of main vasculogenesis related genes.
We demonstrated that using MSCs of different origin resulted in vascular networks with distinct phenotypes. Both types of MSCs supported formation of mature and interconnected microvascular networks. However, BMSCs induced formation of fully perfusable microvasculature with larger vessel area and vessel length compared to ASCs. Co-culture with ASCs resulted in only partially perfusable microvascular networks. Immunostainings revealed that BMSCs had greater potential to differentiate towards pericytes than ASCs. The gene expression analysis revealed significant differences in the expression of endothelial-specific and pericyte-specific genes, as well as genes involved in vasculature maturation and remodeling.

Overall, our study provides valuable knowledge on the properties of BMSCs and ASCs as vasculature supporting cells and highlights their distinct directing role in the regulation of microvascular phenotype that might have implications in bone tissue engineering applications.

keywords: In vitro vascularization, organ-on-a-chip, mesenchymal stem cells, endothelial cells

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EXTRACELLULAR VESICLES SECRETED BY OSTEOGENIC-DIFFERENTIATED MESENCHYMAL STEM CELLS PROMOTE BONE FORMATION IN RAT CALVARIAL DEFECT

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Background:
Mesenchymal stem cell (MSCs) -based therapy has a promising potential in bone tissue regeneration. Although, growing evidence has suggested that paracrine mechanisms may be involved in the underlying mechanism of MSC transplantation, and extracellular vesicles (EVs) are an important component of this paracrine role. However, information on the influence of different microenvironmental stimuli of MSCs culture conditions on the osteogenic effects of EVs is scarce. The main purpose of this study was to determine whether EVs derived from MSCs under normoxic (Normo-EVs), Hypoxic (Hypo-EVs) and chemically osteogenic induced MSCs (Osteo-EVs) show greater effects on osteogenic differentiation potential in vitro and on the bone formation of calvarial defects in vivo, and whether findings are associated with various proteins profile.

Methods:
Undifferentiated MSCs were incubated under normoxic and hypoxic culture conditions, and 7-days of chemically osteogenic induced MSCs were incubated under normoxic conditions, for 72 h. Conditioned media were collected and concentrated onto 100 kDa centrifugal filters (UF), followed by separation of EVs using size-exclusion chromatography method (SEC). The Normo-EVs, Hypo-EVs, and Osteo-EVs recovery were characterized by size distribution using DLS, morphology using TEM, and flow cytometry analysis of tetraspanin CD63 and CD81. The proteomic composition of different groups of EVs was characterized by LC-MS/MS. We evaluated the in vitro effect of EVs groups (10 µg/ml) on the proliferation, scratch assay, and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hMSCs) by Alkaline phosphatase (ALP) staining, Alizarin red S (ARS) staining, and qRT-PCR for osteogenic related genes, respectively. Furthermore, Osteo-EVs (50 µg/ml) were combined with collagen membrane scaffolds (MEM) to repair critical-sized calvarial bone defects in rats, and the efficacy was assessed using in vivo and ex vivo µCT, and histological examination. The in vitro release of Osteo-EVs from MEM scaffolds and their internalization by cultured MSCs were also examined.

Results:
Using UF-SEC, we could isolate and characterize EVs from all groups. We found that all EVs groups could profoundly enhance the proliferation, and migration of cultured hMSCs. However, Osteo-EVs were shown greater effects on the in vitro osteogenic differentiation of hMSCs as detected by higher mRNA expression levels of late markers of osteogenesis-related genes, BSP and OC, and calcium deposit using ARS. In addition, Osteo-EVs/MEM combination scaffolds could enhance greater bone formation after 4 weeks as compared to native MEM loaded with

serum-free media. In vitro assay showed that the Osteo-EVs could release from the MEM scaffold and could be internalized by cultured hMSCs.

Conclusions:
We suggest that EVs derived from chemically osteogenic induced MSCs can significantly enhance both the osteogenic differentiation activity of cultured hMSCs and the osteoinductivity of MEM scaffolds. These results indicate that Osteo-MSC secreted nanocarriers-EVs combined with MEM scaffolds can be used for repairing bone defects.

94238130844

DEVELOPMENT OF ANGIOGENIC BIOINK FOR VASCULARIZED BONE TISSUE ENGINEERING

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Introduction
Endothelial cells (ECs) have potential in bone tissue engineering due to their important role on vascularization. For repairing bone defects, co-culturing of ECs and bone marrow stem cells (BMSCs) is suggested to induce both the capillary network formation and bone regeneration. The communication between the two cell types has been experimented on settings based on cell number ratio, culture medium [1-2] and culture distance [3]. With regards to 3D printing technology, EC suspensions have been previously flushed into engineered lumens or canals to initiate vessel formation in existing structures [4-5]. However, bioprinting is yet a rarely used form of technology for delivering ECs. Vascu-ink, containing fibrinogen and gelatin, aimed for soft, elastic and dynamic 3D environment, enabling the rapid maturation of bioprinted ECs. The aim of this study was to investigate the influence of a multimaterial bioink, vascu-ink, in a co-culture setting on angiogenic tissue maturation.

Methodology
The performance of the vascu-ink with ECs was characterized as single bioink but including the co-culture effect by seeding BMSCs outside the bioink structures. To investigate the potential of vascu-ink as an EC carrier, the bioink was mixed with the ECs (1x107/ml) and bioprinted in a four-layered structure using a 3D-Bioplotter (EnvisionTEC) with a 250 µm metal needle. The structures were crosslinked externally with thrombin (2.5U/ml) and CaCl2 (100mM). BMSCs were seeded on the bottom of the well plate and the crosslinked vascu-ink samples were lifted on top. The co-cultures were then followed for structural integrity, cell viability (Live/Dead staining), metabolic activity (Cell Counting Kit -8) and tubular formation, via immunostaining, for up to 21 days. Endothelial growth media was used for the first three days and then switched into osteogenic media. After that, the media was changed three times a week.

Results
The developed vascu-ink was very compliant as desired. The viability of the ECs was high throughout the culture period, cells were spreading and migrating, and the metabolic activity of ECs was maintained with the BMSCs. The printed structures survived the culture period but had gradually lost their fidelity as the material was preferred by both of the cell types. The desired tubular formations and organization of the ECs was recorded by both Live/Dead staining and immunostaining.

Conclusions
The promising results indicate that the developed bioink serves as a tissue specific cell carrier and culture environment. The co-culture of the two cell types was also beneficial in tubular formation.

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keywords: 3D bioprinting, bioink, vascularization, bone tissue engineering

52354540746

MACROPHAGE MEDIATED IMMUNOMODULATION BY EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS COMBINED WITH BIPHASIC CALCIUM PHOSPHATE GRANULES FOR BONE REGENERATION

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INTRODUCTION:
Mesenchymal stromal cells (MSC) combined with biphasic calcium phosphate biomaterial (BCP) is a promising clinical strategy to repair and regenerate lost bones [1]. Further, MSC derived extracellular vesicles (MSC:EV) are established factors of paracrine inter-cellular communication with various cell types, including immune cells, which impacts their regenerative potential [2] . However, scarce studies have explored the immune modulation behavior of EV isolated from MSC+BCP constructs (MSC+BCP:EV). The aim of this study was to isolate and characterize MSC+BCP:EV, including from inflammatory primed cells (MSCp+BCP:EV).Isolated EVs from all MSC groups were exposed to primary human macrophages to determine change in macrophage maturation and polarization states.

METHODS:
EV isolation was done by using size exclusion chromatography (SEC) columns (PURE-EV, HansaBioMed Life Sciences Ltd., Estonia). After confirming the particle size in eluted EV fractions via dynamic light scattering (DLS) and protein amount by BCA, fraction no. 8-15 were selected for further analysis. EVs were characterized by TEM and flow cytometry. A human cytokine 27-plex assay was used with a Bio-Plex® 200 System (both from Bio-Rad Laboratories) to measure the cytokine content of EVs. Macrophages were obtained from differentiation of primary blood derived monocytes, which were isolated from donor buffy coats via magnetically activated cell sorting (MACS, Miltenyi Biotec GmBH).

RESULTS:
Size and morphologies of EVs from different MSC system were found comparable. Further, MSC+BCP:EV showed less proinflammatory, whereas MSCp+BCP:EV showed more immunomodulatory and angiogenic cytokine profile compared to MSC:EV. Functional macrophage analysis revealed increased potential of MSCp+BCP:EV to induce unpolarized/naive macrophages (M0) into an anti-inflammatory phenotype (M2), as compared to EVs from an unprimed construct (MSC + BCP:EV) and MSC alone (MSC:EV). Further, it was found that MSCp + BCP:EVs also have an enhanced potential for bi-directional macrophage polarization switching (i.e., from pro- to anti-inflammatory state and vice-versa).

CONCLUSIONS:
This study established methods to isolate and characterize EVs from a MSC and biomaterial constructs. We showed that both priming and biomaterials have differential effect on EV-cytokines and hence immunomodulation by EVs. EVs derived from primed MSC + BCP constructs showed enhanced potential to modulate both naïve (M0) and pro-inflammatory (M1)

human macrophage subsets towards an anti-inflammatory (M2) type. Such properties were attributed to the higher levels of immunomodulatory cytokines present in the MSCp + BCP derived EVs. Thus, our study provides new insights into role of EVs in MSC-biomaterial induced bone regeneration [3].

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keywords: Mesenchymal stromal cells, biphasic calcium phosphate, biomaterial, extracellular vesicles or EV, macrophages, immunomodulation, macrophage polarization, cytokines, bone regeneration

94238122617

THE INFERIOR IN VIVO OSTEOGENICITY OF HUMAN MSC FROM ADIPOSE TISSUE COMPARED TO BONE MARROW IS CORRELATED WITH HIGHER IMMUNE RESPONSE WITHIN TISSUE ENGINEERED CONSTRUCTS

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Introduction:
Adipose tissue-derived stem cells (ATSCs) have been used as an alternative to bone marrow mesenchymal stem cells (BMSCs) for bone tissue engineering. However, several studies have reported that the efficacy of ATSCs in bone regeneration in comparison with BMSCs remains inferior 1,2. The aim of this study was to investigate the mechanism underlying differences in ATSCs versus BMSCs osteogenicity in tissue-engineered constructs by focusing on the innate immune response mediated by the ATSCs.

Methodology:
Bone formation induced by transplanted human BMSCs and ATSCs combined with calcium carbonate ceramic granules (named tissue constructs) was evaluated in vivo in an ectopic mouse model. Explants were analyzed by µCT and histology. Kinetic analyses of both the expressed human and murine genes pertaining to osteogenesis, angiogenesis, and inflammatory response in tissue constructs explanted at 0, 7, 14, and 28 days post-implantation were performed. The gene expression and secretome profiles of pro-inflammatory cytokines/chemokines in both ATSC and BMSC were analyzed.

Results:
All constructs containing BMSCs induced ectopic bone formation and histological observation of explants revealed the presence of new bone with the presence of osteoclastic (TRAP +) multinucleated cells on contact with the ceramic particles. On the contrary, the constructs containing ATSCs did not generate (or minimally) bone tissue; they were infiltrated with fibrous tissue, and numerous TRAP- multinucleated giant cells (MNGC) were observed. Gene expression analysis of explants revealed that implanted human BMSCs differentiated into the osteogenic lineage in vivo concomitantly with the osteogenic differentiation of host murine progenitors. In contrast, the osteogenic differentiation in construct-contained ATSCs started later than in BMSCs, when only less than 1% of implanted ATSC were present; no osteogenic differentiation in host murine cells occurred. Expressions of genes pertaining to vascularization were not significantly different between both groups. Regarding the inflammatory response, compared with BMSCs, the expressions of human IL1b and IL6 genes were highly upregulated in implanted ATSCs during the first-week post-implantation and then decreased; In parallel,

murine IL1b was also upregulated in ATSC-containing constructs as were the M1/MNGC-associated iNOS and CD86 murine genes. An extensive analysis of gene expression of human cytokines and chemokines comparing the ATSC and BMSC contained in constructs at day 0 (before implantation) was conducted. This revealed up-regulation of 23 inflammatory mediators out of 84 tested in ATSC compared to BMSC (the highest (> 30-fold) upregulated genes were CSF3, CXCL10, CXCL5, CXCL11). Only CXCL12 (SDF1), RANKL, and BMP4 were slightly (3-7 fold) upregulated in BMSCs. Such a pro-inflammatory profile of ATSCs was confirmed at the protein level after quantification in the construct-contained cell supernatant.

Conclusion:
In contrast to BMSCs, ATSCs display no/weaker osteogenic potential in vivo. ATSCs are a transient source of proinflammatory cytokines and chemokines that promote an inflammatory environment within the cell-containing constructs. This event correlates with impaired osteogenic differentiation of both implanted ATSCs and host osteoprogenitors.
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keywords: mesenchymal stem cells, tissue source, bone formation, immune response

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FLUID-FLOW MEDIATED CYTOSKELETAL ADAPTATION REGULATES THE GROWTH AND FATE OF BONE MARROW MESENCHYMAL STEM CELLS

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Introduction:
Mesenchymal stem cells (MSC) regulate their behavior by sensing mechano-environmental factors.
Accumulated evidence indicates that appropriate mechanical force including fluid shear stress enhances the osteogenic property of MSC on 3D polymeric scaffolds even without the presence of osteogenic cocktail (i.e., dexamethasone and beta-glycerophosphate). However, despite a common understanding of how cytoskeleton transmits mechanical stimuli, a detailed role of cytoskeleton in fluid flow-induced osteogenesis is not fully understood. The aim of the present study was therefore to assess cytoskeletal modulation under fluid force and then explore causal relationship with altered cell growth and flow-induced osteogenic differentiation by using a perfusion bioreactor.

Methodology:
MSC from Lewis rat bone marrow (rBMSC) were seeded on 3D microporous scaffolds made of Poly(L-lactide-co-trimethylene carbonate) and subjected to laminar flow excreting shear stress at 1 mPa on an average for 14 days in a perfusion bioreactor. Cytoskeletal modulation was assessed by, but not limited to, RT-qPCR array, cell morphological analysis, enzymic activity of Rho-associated protein kinase (ROCK), and level of phosphorylation of myosin light chain. Transcriptional profile of osteogenesis-related markers in rBMSC under fluid stimuli was compared with that induced by the osteogenic cocktails and a static control without osteo-inducement. To evaluate the role of cytoskeletal modulation in flow-induced osteogenesis, pharmacologic inhibition of cytoskeletal modulators, namely, Rho GTPases, ROCK, myosin light chain kinase and non-muscle myosin II ATPases, was performed to induce cell relaxation during perfusion culture. With the inhibitors, cell growth and osteogenic differentiation were further evaluated.

Results:
Under the fluid flow, rBMSC significantly altered the expression pattern of mRNA related to cell morphogenesis and focal adhesion including Pkt2, Prkca, Rock1 and Rock2. This was accompanied with cell morphological alternation characterized by cell contraction and actin stabilizayion, and the enhanced phosphorylation of myosin light chain was observed. In such a condition, rBMSC upregulated a number of osteogenic markers including Runx2, Sp7, Col1a1, Bmp2, ALPL and Spp1. Interestingly, the mRNA expression pattern of osteogenic markers differed from dexamethasone-induced osteogenesis. The inhibition of cytoskeletal modulation cascade from Rho activation to actomyosin contraction at different levels suppressed the flow-induced upregulation of the osteogenic markers. Despite the fact that cell proliferation was suppressed by fluid flow, the inhibition aggravated cell growth even further while the inhibitors

did not show a notable suppressive effect on proliferation in the static control.

Conclusion:
The present study using a perfusion bioreactor demonstrated that rBMSC responded to a low level of fluid stimuli by cytoskeletal moduration, which was associated with altered cell growth and osteogenic differentiation on 3D polymeric scaffolds. The inhibition of cell contraction revealed that cytoskeletal modulation under fluid stimuli was required for maintaining the proliferative state while it directed rBMSC fate towards an osteogenic lineage.

comment:Symposium: New insights underlying mesenchymal stem cell-mediated bone regeneration

keywords: Dynamic cell culture, Bioreactor, mesenchymal stem cells, osteogenic differentiation, cytoskeletal modulation

S48
Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy
Room: S4 A
(29 Jun 2022, 11:00 - 12:30)

Conveners: Bryan Falcones, Joanna Idaszek;
Elena Della Bella

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MICROPATTERNED SURFACES FOR CONTROLLING STEM CELLS MORPHOLOGY AND FUNCTIONS

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Cell morphology plays an important role in controlling cell functions. Application of micropatterned surfaces in cell biology provides reproducible cell morphology and relative stable adhesion and cytoskeleton pattern for investigation of stem cell functions. We have used photo-reactive polymers and UV lithography to prepare micropatterns to control cell size, shape, adhesion area, aspect ratio and chirality to investigate their influences on stem cell differentiation and gene transfection. In this study, the independent influence of adhesion and spreading area on differentiation of human bone marrow-derived mesenchymal stem cells (MSCs) was investigated by using micropatterned surfaces to precisely control cell adhesion and spreading areas.

The micropatterns were prepared by micropatterning non-adhesive PVA on cell adhesive TCPS surface. Ten micropattern structures were designed and prepared to control cell adhesion area and cell spreading area separately. The micropatterns were composed of many TCPS microdots having a diameter of 2 µm in a round circle having a diameter of 70, 60 and 50 µm. The TCPS microdots and round circles were surrounded by PVA. The micropatterns were designed to control the cells to have the same spreading area but different adhesion area, or to have the same adhesion area but different spreading area. MSCs were cultured on the micropatterns. The formation of FAs and the cytoskeletal organization in the cells were investigated to evaluate cell adhesion and spreading state. The mechanical properties of micropatterned cells and the transduction of cytoskeletal force into nucleus were characterized to reveal the mechanism of the influence. The osteogenic and adipogenic differentiation of MSCs on the micropatterned surfaces were evaluated.

When cell spreading area was the same, cells with small adhesion area formed FAs at cell edge. Their cytoskeletal structure was mainly composed of radically assembled DSFs. The lack of myosin binding to DSFs resulted in low cytoskeletal tension. And the YAP/TAZ mainly distributed in cytoplasm. Therefore, cells with small adhesion area preferred to differentiate into adipocytes. Increasing in cell adhesion area reinforced the cell/material adhesion strength. Cells formed integrated actin network including VSFs, DSFs and TAs. Association of myosin with VSFs and TAs generated high cytoskeletal tension. The cytoskeletal tension stimulated accumulation of YAP/TAZ into nucleus. Cells with large adhesion area showed high potential to become osteoblasts. When cell adhesion area was the same, changing spreading area did not significantly affected stem cell fate determination. Cells with the same adhesion area showed similar potential of osteogenic or adipogenic differentiation. The results indicated that the adhesion area rather than spreading area played more important roles in manipulating stem cell functions. Large adhesion area facilitated the osteogenic differentiation, while small adhesion area promoted the adipogenic differentiation.

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HYALURONIC ACID BASED NEXT-GENERATION BIOINK FOR 3D BIOPRINTING OF A HUMAN STEM CELL DERIVED CORNEAL STROMA EQUIVALENT AND A 3D CORNEA TISSUE MODEL WITH INNERVATION

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Introduction
There is a dire short of donor corneas for cornea transplantation, leaving millions of visually impaired patients without treatment1. 3D bioprinting holds tremendous potential for fabrication of cornea mimicking structures. One of the key technological challenges in 3D bioprinting is the establishment of bioink compositions that allow both ideal printability as well as biocompatibility. To address these needs, we developed a hyaluronic acid (HA)-based bioink for 3D bioprinting of cornea tissue engineering (TE).

Methodology
HA-based bioink was prepared using hydrazone crosslinking chemistry2. Crosslinking components were combined with rheological modifiers to obtain a printable bioink. The shear thinning property and viscosity of the bioink as well as the mechanical stability of the printed structures were determined with a rheometer. Extrusion-based 3D bioprinting was used. The shape fidelity and self-healing properties of the bioink were explored. Human stem cells, such as human adipose stem cells (hASCs) and hASC-derived cells were chosen for printing cornea stroma equivalents. The printed constructs were evaluated for their cell viability, proliferation and microstructure with LIVE/DEAD® and PrestoBlue™ viability assays, immunofluorescence (IF) and hematoxylin and eosin stainings. Key protein expression was determined with IF and quantitative PCR. Moreover, 3D printed stromal equivalents were implanted into ex vivo porcine corneal organ cultures to explore integration to host tissue. Finally, human pluripotent stem cell derived neurons (hPSC-neurons) were 3D bioprinted to the periphery of the cornea stroma equivalents, and the integration of neuronal extensions to the printed structures was explored.

Results
The developed HA-based bioink showed excellent shear thinning property, viscosity as well as printability. Quality prints with high-resolution and good shape fidelity were achieved. Moreover, HA-DA bioink discs showed self-healing after 24 hours of healing. Importantly, HA-based bioink demonstrated excellent biocompatibility with all explored human stem cells and human stem cell derived cells. Cells in printed structures showed good tissue formation seen with positive expression for connexin 43 and formation of cellular networks. Corneal stroma equivalents with appropriate cell organization and positive expression of lumican were successfully

manufactured. Moreover, 3D bioprinted cornea stromal equivalents demonstrated excellent integration to host tissue in ex vivo organotypic cultures after 21 days. While inspecting the innervation of cornea stromal equivalents in 3D bioprinted cornea tissue model, the printed structures with HA-based bioink allowed the ingrowth of long neuronal extensions. Target cells in the cornea stromal equivalents accelerated the neuronal extension growth compared to printed structures without cells.

Conclusions
We have developed a HA-based bioink using click chemistry that fills the demands of next-generation bioinks with excellent printability, stability, biocompatibility as well as tissue formation. Here, we demonstrated that the developed bioink is feasible for 3D bioprinting of cornea stroma equivalents. Moreover, we manufactured the first 3D bioprinted cornea tissue model with innervation. The developed bioink and the printed human stem cell derived cornea stromal equivalents hold great potential for future cornea TE applications.

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keywords: 3D bioprinting, bioink, cornea, human stem cell, tissue engineering

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DEVELOPMENT OF AN IPSC LOADED BIOMIMETIC SCAFFOLD SYSTEM FOR SPINAL CORD APPLICATIONS

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Following spinal cord injury, a complex scar forms around a lesion cavity, preventing axonal regrowth. Despite ongoing development of stem cell treatments for spinal cord injury, effective repair of the cord remains a challenge in part due to the lack of a supportive environment and cell death. Therapeutics that physically bridge the cavity with a neurotrophic environment while simultaneously delivering stem cells to restore lost tissue may have potential. Building off success in our lab in peripheral nerve repair[1], we aimed to identify neurotrophic extracellular matrix (ECM) proteins to develop novel, biomimetic functionalized hyaluronic acid scaffold implants and to determine their trophic capacity for spinal cord applications using multiple cell models[2]. By optimizing scaffold stiffness and matrix composition for stem cell delivery, it was hypothesized that the pro-regenerative signaling properties of trophic induced pluripotent stem cell (iPSC) derived astrocyte progenitors could be enhanced by scaffold physiochemical properties to promote cord repair.

To identify neurotrophic ECM proteins, astrocytes and various neuronal cells were cultured on a range of ECM combinations to identify a novel neurotrophic substrate. Following incorporation of the neurotrophic substrate mix into freeze-dried 3D hyaluronic acid scaffolds of varying stiffness, scaffold physicochemical properties were characterized. Astrocytes, neurons, dorsal root ganglia (DRG) and iPSC-derived astrocyte progenitors were cultured in scaffolds up to 21 days and the effect of scaffold stiffness and matrix composition was assessed. Additionally, the impact of scaffold properties on the therapeutic effectiveness of iPSC-derived progenitors was assessed using various models.

Screening of central nervous system ECM components revealed that a combination of collagen-IV (Coll-IV) and fibronectin (FN) synergistically enhanced neuronal and astrocyte outgrowth compared to control substrate poly L-lysine. Subsequently, hyaluronic acid scaffolds functionalized with Coll-IV/FN were manufactured using different concentrations of hyaluronic acid to produce scaffolds of varied stiffnesses ranging from soft to stiff (0.8-3kPa). Astrocytes cultured in soft, Coll-IV/FN functionalized scaffolds, increased secretion of IL-10 and exhibited morphologies typical of resting phenotypes. Furthermore, soft CIV/FN scaffolds significantly enhanced neurite outgrowth from DRG explants (a model of axonal growth) compared to stiffer scaffolds. Soft, but not stiff Coll-IV/FN scaffolds also promoted iPSC progenitor infiltration, differentiation and glutamate uptake (a measure of functional capacity) while encouraging iPSC-derived spheroid growth. Furthermore, conditioned media taken from soft, CIV/FN iPSC-loaded but not stiffer scaffolds significantly enhanced neurite outgrowth 2.8 fold. Finally, mouse spinal

cord and DRG explants cultured on soft, Coll-IV/FN iPSC scaffolds promoted astrocyte migration and long axonal extensions between DRG and iPSC neurospheres within scaffolds.

These data indicate that by appropriately tuning the physicochemical properties of scaffolds to mimic that of the uninjured spinal cord, significantly enhances astrocyte responses while promoting neurite extension. Furthermore, biomimetic scaffolds promote the paracrine activity of patient-derived progenitor cells, enhancing their therapeutic capacity. Overall, the impact of biomaterial properties on the therapeutic effectiveness of stem cells has significant implications for spinal cord repair applications.

This work is funded by the IRFU-Charitable Trust, Anatomical Society and SFI-AMBER centre.

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keywords: ‘Spinal Cord Injury’, ’Scaffold’, ‘Induced Pluripotent Stem Cell (iPSC)’, ‘Neurons’, ‘Astrocytes’

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ROAD TO UNIVERSAL ORGANS: DECELLULARIZED LIVER REPOPULATION WITH HLA I-II KNOCKOUT HEPATOCYTES IN A DYNAMIC BIOREACTOR CULTURE

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Introduction:
The past few years saw an increasing trend in liver disease prevalence [1]. Orthotopic transplantation is the current gold standard treatment. However, its practice is affected by strong complications represented by the paucity of available organs and the necessity of long-term immunosuppressive therapies. Tissue engineering strategies represent suitable alternatives by combining scaffolds matrices and patients' autologous cells. In this setting, CRISPR/Cas9 genome edited HLA knockout human induced pluripotent stem cells (hiPSCs) represent a promising cell candidate for a universal strategy, conjugating high cell availability and wide tissue-specific differentiation capacity [2]. The present work aims to rebuild a functional liver substitute seeking for the definition of a promising strategy to evade T cell surveillance upon in vivo transplantation.

Methodology:
hiPSCs underwent CRISPR/Cas9 genome-editing to prevent expression of HLA class I and II. HLA Class I/II-/- hiPSCs were differentiated according to standard protocols to obtain definitive endoderm (DE), hepatic endoderm (HE) and immature hepatocytes (IH) cells in a 2D monolayer [3]. hiPSCs-IH were transferred to Matrigel® 3D culture in hepatic organoid expansion medium to generate self-replicating hepatic organoids [3]. To obtain decellularized scaffolds, mouse livers were cannulated via portal vein and decellularized via already established detergent-enzymatic treatment [4]. 17 M cells were obtained from enzyme mediated organoid disaggregation and were further seeded in the decellularized scaffold via the portal vein. Repopulated livers were cultured in a custom-designed bioreactor in a dynamic condition provided by peristaltic pump (flowrate=0.5 ml/min). Constructs were cultured for 7 days in hepatic organoid expansion medium and further supplemented with Oncostatin-M and Dexamethasone to induce terminal

hepatocyte differentiation for the following 7 days [5]. Histological and immunohistochemistry analysis were performed to study scaffold repopulation and expression of hepatic maturation and biliary polarity markers. qRT-PCR analysis was performed to analyse the expression of mature hepatic markers including cytochrome 3A4,1A2 (CYP3A4, CYP1A2), hepatocyte nuclear factor 4 alpha (HNF4α).

Results:
Complete HLA double knockout was confirmed by genome-sequencing analysis.
H&E staining showed successful scaffold repopulation supported by the bioreactor. Immunofluorescence analysis revealed the expression of mature hepatocytes markers (HNF4α, Alpha1-anti-trypsin, human albumin) together with biliary polarity markers such as zonula occludens 1 (ZO1) and multi-drug resistance protein 2 (MRP2), proving a nearly complete mature state. HLA class I and II expression were not detected in immunofluorescence staining, supporting sequencing analysis. qRT-PCR results showed enhanced expression of HNF4α, CYP3A4 and CYP1A2 in the bioreactor culture compared to the static in vitro control, highlighting the effect of the scaffold environment and the dynamic culture on hepatic maturation.

Conclusion:
Universal therapeutic strategies are mostly unavailable for the majority of end-stage diseases, including liver diseases. The present work demonstrated the development of a universal functional liver graft by combining organotypic acellular scaffolds and universal hepatocytes obtained from HLA class I/II-/- hiPSCs. The decellularized 3D liver microenvironment efficiently supported hiPSCs-derived hepatocytes engraftment and proliferation. This experimental evidence proves that universal hiPSCs represent a valid candidate to be employed in "ready-to-use" tissue engineering and regenerative therapies, with the promise to overcome immune rejection upon graft transplantation.

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keywords: hiPSCs, HLA class I II knockout, liver bioengineering, organoids, bioreactor

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MULTIFUNCTIONAL 3D BIOPRINTING FOR TISSUE INTERFACES

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Recent progress in 3D bioprinting technologies has shown promising results for the multi-material design of interface tissue engineering. However, the achievement of structural integrity between tissues with different mechanical strengths is a great challenge in the bio-fabrication of tissue interfaces, such as bone-cartilage interfaces1. In this study, we used multimaterial 3D bioprinting approach for the deposition of hydrogel structures based on an aspiration-on-demand capillary method. The obtained construct showed a high degree of structural integrity at the interface between adjacent ink segments mimicking the bone-cartilage tissue interface. To do so, specific amounts of different cell-laden hydrogels were extruded in the same capillary and deposited at the desired geometrical pattern2. The ink segments, comprised of Alginate (Alg)/ Gelatin (Gel), and Carboxymethylcellulose (CMC)/ Gelatin for soft and hard tissue, respectively. The inks were prepared as the following: Alg and CMC were dissolved in PBS separately by forming amide bonds by carbodiimide-mediated precipitation of material's carboxyl groups with Tyramine's amino groups utilizing EDC/NHS reaction. Therefore, a specified amount of tyramine and NHS were applied to the CMC and Alg mixture followed by adding EDC was added to the NHS at an equal molar ratio to make Alg-Ph and CMC-Ph. Two polymer mixtures were stirred for 24 h, dialyzed against deionized water, and were lyophilized for 3 days. Gelatin was added at 15% w/v in PBS and incubated for 1 day at 37 °C to acquire Gel-Alg-Ph and Gel-CMC-Ph. The hydrogels were crosslinked at the presence of ruthenium (Ru) mixture and sodium persulfate (SPS) photo-initiating system under a visible light source at the concentration of 0.1/1 Ru/SPS (mM/mM). Mesenchymal stem cells (MSCs) were grown and added to the two ink mixtures in various cell counts for the targeted tissue. Mechanical properties of the casted samples were assessed with different parameters such as various Alg-Ph, CMC-Ph, Ru/SPS concentrations, and the visible light exposure time. Differentiation of MSC cells expected after 21 days, histology, immunohistochemistry, and a live/dead test for the bioprinted structure were carried out. [ASF1] The outcomes of the in vitro biochemical studies were corroborated by the result of the histological staining. This research, which investigated the multilayered and hierarchical architecture of osteochondral tissue using 3D biofabricated material compositions, could lead to an improved regeneration of osteochondral lesions in the future.

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keywords: 3D bioprinting, multifunctional bioprinting, tissue interfaces, visible-light crosslinking, Alginate/Gelatin-Carboxymethylcellulose bioinks

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LUNG TISSUE TYPE SELECTED AMNIOTIC FLUID DERIVED MESENCHYMAL STEM CELLS FOR TREATMENT OF BLEOMYCIN INDUCED PULMONARY FIBROSIS IN A RAT MODEL

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Introduction
Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fatal form of diffuse interstitial lung disease which is associated with substantial mortality and a median survival of 3 years from the time of diagnosis. Acute exacerbations (AE) of IPF have been defined as acute, clinically significant respiratory deterioration of unidentifiable cause. Available data suggest that 46% of deaths in IPF are preceded by AE, and the median survival of patients with IPF who experience AE is approximately 3.5 months. Mesenchymal stem cells (MSC) derived from term amniotic fluid (TAF) are neonatal and can be propagated to extremely high amounts for use in cell therapy. TAF-MSC are derived from different tissues of the fetus. Therefore, sorting strategies using tissue-specific markers can prepare a sorted population of cells from each tissue-type. We hypothesize that lung-specific MSC can be used to reduce the overall severity of AE-IPF and lower the risk of mortality due to the pharmacological effects (anti-inflammatory, immunomodulatory, regenerative, proangiogenic, and antifibrotic) these cells may have.

Methodology
RNASeq data from TAF-MSC clones was used to identify tissue-specific markers present on these MSC. Several prospective lung markers were tested by flow cytometry of cultured TAF-MSC. One of these markers was used for cell-sorting using Tyto MACSQuant cell sorter and the positive cell population was expanded to passage 4 (TAF-lung-MSC). Rats were instilled intratracheally with bleomycin to induce fibrosis at day 0. At Day 4 TAF-lung-MSC or vehicle was administered by an IV injection. On day 28 lungs were prepared for histology, stained and scored.

Results
After collection, processing and expansion of TAF cells in a defined media system, different subtypes of MSC were identified. Of these, one type had unique RNA expression and cell surface phenotype expression profiles. This signature was partially similar to MSC collected from lung tissue. After identifying lung-specific clones, candidate marker genes were identified. The validity of the surface marker genes was established using flow cytometry with antibodies directed against the indicated lung surface markers. One of the prospective markers was used for lung-MSC sorting. The positive fraction was further propagated until passage 4 when it was used to treat bleomycin induced pulmonary fibrosis in a rat model. TAF-lung-MSC showed anti-fibrotic effect with significantly less fibrosis in the lungs of rats at 24 days after infusion of MSC than in the bleomycin-treated control group ($p<0.05$) as assessed both by histopathological evaluation (percent parenchyma affected) and fibrosis scoring using the Modified Ashcroft scale. Further, at the termination of the study on Day 28, TGF-β plasma levels were higher in bleomycin-treated control rats than in rats treated with TAF-lung-MSC. No adverse reactions from cell injections were reported. No remaining TAF-lung-MSC could be detected after Day 24, supporting that MSC were cleared from the rats after exerting their effect.

Conclusion
Several markers were found to be good lung-specific markers for sorting of TAF-MSC using the

Tyto MACSQuant cell sorter instrument. Sorted cells were further propagated and TAF-lung-MSC were effective in reducing lung fibrosis in a rat bleomycin induced pulmonary fibrosis model.

keywords: fibrosis, tissue specific, msc, amniotic fluid

73296328146

INTERPLAY BETWEEN ADIPOSE-DERIVED STEM CELLS AND INFLAMMATORY MEDIATORS: IMPACT ON NEURITE OUTGROWTH AND VASCULAR MORPHOGENESIS

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Introduction:
Spinal cord injury (SCI) is a condition that hampers the communication between the brain and the body, resulting in several comorbidities that decrease the patient's life quality. The limited regeneration after SCI is mainly attributed to the injury complexity composed of several interconnected mechanisms. Although reestablishment of lost nerve tracts is essential for functional recovery, it is also important to restore the destroyed blood vessels. Adipose-derived stem cells (ASCs) have been addressed as therapeutic agents do their ability to modulate several repair processes, among neuronal and vascular growth, both at a paracrine and non-paracrine level. However, until now, ASCs did not elicit a total satisfactory repair. To enhance the regenerative features of ASCs, several approaches are being explored. Inflammatory pre-conditioning has shown to augment the anti-inflammatory properties of these cells, but with few reports showing the effects at a neuronal and vascular level.
Therefore, we aimed to understand the impact of pro-inflammatory (LPS+IFN-γ) and pro-regenerative molecules (IL-10) on the neuroregenerative and angiogenic potential of ASCs at three levels: indirect contact, secretome and direct contact.

Methodologies:
The neuroregenerative potential was evaluated by analysis of the neurites produced by rat dorsal root ganglia (DRG) explants. The angiogenic potential was assessed by plating human umbilical vein endothelial cells (HUVECs) on a matrigel matrix, with further morphological analysis of the formed vessels. Each assay was adapted to the type of cell communication studied. Gene analysis for several vascularization and axonal growth-related molecules was

performed after ASCs stimulation.

Results:
ASCs induced neurite growth in all DRG assays. In the secretome and direct contact assay, the control did not induce any growth. No differences between inflammatory stimulations were found regarding neurite area, length and arborization pattern, in any DRG assay. LPS+IFN-γ enhanced the vascular potential of ASCs secretome, forming more vessel-like structures with higher average length and interconnectivity. This effect was lost in the indirect co-culture, with no differences between stimulations at any level. IL-10 did not alter ASCs behavior. Inflammatory stimulation led to alterations in ASCs gene expression, but without a clear shifted phenotype.

Conclusion:
Overall, this work showed that ASCs and their secretome can modulate neurite and vascular processes essential for successful CNS regenerative applications. Furthermore, pro-inflammatory stimulation enhanced secretome angiogenic properties, holding great potential to enhance ASCs therapeutic properties.

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comment:Nuno A. Silva and António J. Salgado share senior authorship

keywords: Spinal cord injury, Adipose-derived stem cells, Inflammatory pre-conditioning, Neurite outgrowth, Vascular morphogenesis

52354519528

TOWARDS APPLICATION OF CELL THERAPY USING HIPSC-DERIVED MSCS AS A STABLE 'OFF-THE-SHELF' CELL SOURCE

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INTRODUCTION
Therapeutic application of mesenchymal stromal cells (MSCs) has been suggested as a promising regenerative treatment for osteoarthritis (OA) by virtue of their paracrine-mediated chondroprotective and immunomodulatory activity. Nonetheless, collection of MSCs is an invasive procedure and the therapeutic efficacy of autologous MSCs are subject to large variability due to the varying potency of individual MSCs.1 In the current study we therefore set out to explore human induced pluripotent stem cell (hiPSC) derived MSCs (hiMSCs) as sustainable cell sources for OA stem-cell treatment by identifying and characterizing the therapeutic OA-secretome of hiMSC that contribute to a beneficial chondrocyte state.

METHODOLOGY
An established protocol was applied to generate hiMSCs with high similarity to hBMSCs.2 To mimic the inflammatory environment, generated hiMSCs and hBMSCs were exposed to different cytokines (IL6 or a combination of TNFα and IFNγ). RNA sequencing was performed to determine the secretome of licensed hiMSCs (N=6). Data were analyzed in R using DESeq2 package while considering significant differentially expressed genes (DEGs) in comparison to unlicensed controls when FDR<0.05. Luminex was applied to quantify cytokine levels in cell culture media. Effects of licensed hiMSCs on human primary articular chondrocytes (hPACs; N=3, RAAK study) were determined by RT-qPCR following 3 days of co-culture in transwells.

RESULTS
Cell licensing with IL6 resulted in modest changes in gene expression effect sizes and 5 FDR-significant genes. Of note among these genes was SOCS3 encoding suppressor of cytokine signaling 3 (FC=2.0, FDR=2.2x10-2). In contrast, TNFα+IFNγ showed substantial differences as compared to unlicensed cells. Particularly, with respect to well-known immunomodulatory genes such as IDO1 (over 1000-fold induction), MCP1, and HLA-DRA (both around 100-fold induction). Likewise, we demonstrated significant increase of secreted cytokines in the culture media of TNFα+IFNγ exposed hiMSCs. The changes in licensed secretome of hiMSCs were highly comparable to those in hBMSCs, independent of the licensing factor applied, suggesting that the therapeutic mode of action is the same.
Effects of hiMSCs licensed with either IL6 or TNFα+IFNγ were explored in co-cultures with hPACs. The inverse effects of both licensing methods on chondrocytes with respect to expression of COL2A1 and OA markers MMP13, ADAMTS5, CD55 and IL11 suggested that IL6-licensed hiMSCs exerted direct pro-chondrogenic activity.

CONCLUSION
Our results demonstrate that hiPSC-derived MSCs should be considered promising candidates as stable source for application in cell therapy. Particularly, IL6 licensed hiMSCs showed direct chondroprotective effects as reflected by decrease in OA markers. Transcriptome wide analyses

revealed SOCS3 as a candidate effector. Further studies will address whether, in vivo, this may result in additional beneficial effects by virtue of its immune-suppressive activity.
In line with previously determined similarities between characteristics of our hiMSCs and hBMSCs, the current study demonstrates potential of iPSC-derived MSCs in response to inflammatory environment. Findings pave the way to further explore their application in the clinic as off-the-shelve cell source to treat osteoarthritis.

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keywords: cell therapy, immunomodulation, hiPSCs, osteoarthritis

S49
Novel strategies to assess cellular response to biomaterials
Room: S3 B
(28 Jun 2022, 11:00 - 12:30)

Conveners: Carmelo De Maria; Julieta I. Paez

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QUANTUM SENSING FOR MEASURING FREE RADICAL GENERATION IN LIVING CELLS

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Free radical generation plays a key role in many biological processes including cell communication, immune responses and maturation. However, radical formation is also a hallmark of ageing and often elevated when cells experience stress. As a result, they are important in many different diseases including cancer, cardiovascular diseases or viral and bacterial infections. However, free radicals are short lived and reactive and thus difficult to measure for the state of the art. Also, since they occur in low concentrations they are difficult to localise. We have circumvented this problem by using quantum sensing which allows nanoscale MRI. Here I would like to show our work in immune cells [1,2]. In these cells we were able to target nanodiamonds to single mitochondria and measure the metabolic activity of the organelles as well as their stress response[1]. We were able to conduct a similar study also in primary cells which where harvested from donors[2]. Despite these donors all being healthy there were drastic differences in how aggressive their dendritic cells reacted towards a stressor. With these measurements we could confirm that these differences that were also evident in other metabolic parameters also were obvious when observing free radical generation.

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31412714049

A NEW SEMI-ORTHOTOPIC BONE DEFECT MODEL FOR CELL AND BIOMATERIAL TESTING IN REGENERATIVE MEDICINE

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Introduction
Bone defects above a critical size exhaust the self-healing capacity of bone and in these circumstances intervention is needed to promote regeneration. In recent decades, an increasing number of tissue engineered bone grafts have been developed. However, expensive and laborious screenings in vivo are necessary to assess their safety and efficacy. Rodents are the first choice for initial in vivo screens but their size limits the dimensions and number of bone grafts that can be tested in orthotopic locations. Here, we report the development of a refined subcutaneous semi-orthotopic bone defect model coupled with a semi-automated longitudinal in vivo micro-CT registration method1.

Methodology
The model is based on four bovine bone implants, which are subcutaneously implanted on the back of immunodeficient mice, where bone healing potential of grafts can be evaluated in an artificially created 4mm wide defect in vivo. Crucially, these defects are "critical size" and unable to heal within the timeframe of the study without intervention. Various grafts were prepared to modulate bone regeneration inside the defect; cortical bone chips, tissue engineered constructs consisting of MSC pellets, collagen scaffolds or a combination, and cartilage grafts. Animals were

scanned by micro-CT after implantation and then every two weeks until sacrifice at week 8. For analysis of architectural changes in bone structure, a semi-automatic algorithm for longitudinal micro-CT imaging was developed. Micro-CT scans were segmented into binary datasets and afterwards a co-registration method was performed to assess bone morphometric parameters of the implanted defect over time, followed by histological assessment.

Results
Firstly it was assessed if a graft composed of cortical bone chips, the current gold-standard, would increase bone regeneration in the defect of our model. After 8 weeks, empty defects filled their mineralised bone volume, analysed by micro-CT, by 4±3%, while the defects implanted with bone chips showed a net bone volume increase of 26±8%. Histological analysis confirmed that bone formation was stimulated by bone chips. In this study we demonstrated that bone regeneration can be assessed and that osteogenic performance of grafts composed of solely biomaterial, cells or a combination can be studied effectively. Additionally, with the use of image registration a method to analyse the testing pocket only was developed, which was combined with bone morphometric analysis to monitor defect healing longitudinally in the same animal, thus limiting the number of animals needed .

Conclusion(s)
Our novel semi-orthotopic in vivo model suggests that it is possible to overcome some of the current limitations that rodent bone defect models pose, in particular regarding number and size, since in previous mouse critical-size defect models defects with a size of 3mm3 were reported, while in our model each of the four grafts contains a 50mm3 defect. With the semi-automated micro-CT method we have developed a quantitative technique for assessing the testing pocket of our bone defect model. Our data supports that the semi-orthotopic model combined with the novel micro-CT registration method provides an excellent approach for assessment of new biomaterials or tissue engineered constructs for large bone defect repair.

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keywords: Animal model, Guided tissue regeneration, Bone substitutes, Endochondral ossification, Micro-CT analysis

31412714589

MECHANOTRANSDUCTION AND RESHAPING AT THE NUCLEAR ENVELOPE: INVESTIGATING THE LAMIN A/C-SUN1 INTERACTION

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Introduction
Extracellular matrix communicates to the nuclear environment by external stimuli that affect Lamina organization and chromatin distribution [1]. It is known that integrins transmit mechanical stimuli to the actin filaments in turn connected to the LINC complex consisting of Nesprin and SUN proteins [2]. SUN proteins are supposed to link the nuclear Lamina [2], that rearranges according to external impulse. Despite the relevance of SUN1-Lamin A/C in nuclear reshaping, their binding domains have not been identified yet.
We here designed computational protocol to study the SUN1-Lamin A/C binding domain that plays key role in lamina sensitivity to external load.

Methodology
Computational protocol was designed to reconstruct the SUN1 nuclear domain (1-315aa according to Uniprot server). The secondary structure of SUN1 was predicted via a consensus study of eight different servers while I-TASSER server was used for tertiary structure. To stabilize the 3D selected model, molecular dynamic annealing test was implemented via NAMD software (from 300K to 500K deltat=5ns, deltaT=5K explicit water box; Charmrun force field). To predict SUN1-Lamin A/C interaction, Haddock server was used to perform molecular docking analysis between SUN1 predicted model and all the X-ray solved domains of Lamin A/C in order to cover its full length. Combining affinity energy values with cluster dimension we selected the most reliable 3D structure of SUN1-Lamin A/C complex. H-bonds analysis was performed with VMD server.

Results
The secondary structure of SUN1 N-terminal domain was estimated with 87% of confidence value and it was used to predict 3D model. 3D model reliability was supported by 65.4% of similarity with expected secondary structure and its high stability during annealing simulation (90.2% of similarity). Testing SUN1 domain affinity to all the solved Lamin A/C domains we identified the Ig-fold domain (1IFR) as the most affine one due to its high energy interaction as supported by 4 H-bonds (aa 295,303,140,141 and 456,490,496 for SUN1 and Lamin, respectively).

Conclusion
We here estimated SUN1-Lamin A/C interaction structure required to elucidate its key role in force transmission from extra cellular matrix to the nucleus. The reliability of the developed protocol supported by the consistency between our data and the literature ones, introduces our strategy as new promising tool for 3D reconstruction of proteins [3]. Moreover, the SUN1-Lamin A/C complex reconstruction represents the first step in deepen external force effects on nuclear shape. Considering the high occurrence of laminopathies-related single point mutations in Lamin A/C Ig-fold domain, we suggest that these mutations may alter SUN1-Lamin A/C interaction with consequences on nuclear sensitivity and thus gene activation. Further computational analyses could verify this hypothesis and X-ray technique will be used to validate the SUN1-Lamin complex.

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keywords: Lamin A/C, SUN1, structure prediction, molecular docking, mechanostransduction

41883621066

IMMUNE PERFUSION IN CUSTOM BIOREACTORS FOR THE STUDY OF THE EXTRACELLULAR MATRIX-IMMUNE CELL CROSSTALK IN LIVER FIBROSIS

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Introduction
Liver fibrosis is caused by progressive accumulation of extracellular matrix (ECM) coupled with chronic inflammation. Advanced liver fibrosis results in increased risk of liver cancer, cirrhosis, portal hypertension and liver failure, resulting in the need for liver transplantation. Studies of the mechanisms that promote fibrosis are necessary to understand this multi-faceted disease and for the development of novel therapeutic targets. Traditional cell culture models often lack the immune cell compartment and the ECM, failing to recapitulate the complex fibrotic microenvironment, which is highly immune-mediated. Bioengineering allows for the development of disease models to study complex diseases, such as liver fibrosis, where both cellular and extracellular microenvironment components contribute to the pathology. Here, we describe two novel bioengineered models which incorporate the dynamic co-culture of circulating immune cells in decellularised liver ECM-scaffolds, supported by two custom-made perfusion bioreactors, and we demonstrate how these models allow to explore immune responses to fibrotic liver ECMs.

Methodology
We developed two custom-made bioreactors for whole rat livers or human tissue segment culture (WL and HuTS bioreactor respectively). Decellularised normal and fibrotic rat and human liver tissues were obtained following established detergent and enzymatic treatment protocols. Human peripheral blood mononuclear cells (PBMCs) from healthy donors, were perfused in WL or HuTS bioreactors in absence (baseline characterization) or presence of decellularised normal or fibrotic liver ECM-scaffolds. Circulating cell viability, phenotype, and cytokine production were assessed (via FACS and Luminex) in comparison to static culture conditions. The gene expression and phenotype of PBMCs present inside the scaffolds were examined via qPCR and immunofluorescence.

Results
The custom-made bioreactors supported perfusion of PBMCs for up to 7-days without altering cell viability and phenotype in comparison to conventional static culture conditions. Bioreactor culture also improved cell distribution inside the scaffolds compared to static cultures, suggesting that perfusion culture better promotes cell-ECM interactions.
FACS analysis of circulating PBMCs showed that co-culture with both healthy or fibrotic liver matrix-induced an increase in the relative proportion of NKT cells and B cells and that this increase was greater when cultured with fibrotic livers. When cultured with fibrotic scaffolds, the

number of circulating T cells decreased and interestingly, monocytes sub-populations changed in response to healthy or fibrotic liver matrices.
Immunofluorescent staining on sections of perfused ECM-matrices revelated that immune cells infiltrated the ECM-scaffolds. These were mostly composed of monocytes and macrophages, with higher relative proportion in fibrotic livers, indicating fibrotic ECM-induced homing of monocyte-derived macrophages, an event that recapitulates the in vivo fibrotic microenvironment.
Cytokine analysis revealed that the ECM triggers the release of innate and adaptive immune system cytokines and those related to pro-regenerative immune responses.

Conclusion
Here we show the validation of two innovative bioreactor-based systems which allow for the perfusion of immune cells through decellularised liver matrices. This perfusion system proved to be suitable for the study of immune cell interaction with normal or fibrotic ECM, and more in general, with bioengineered multi-cellular liver constructs, and showed that mechanisms of chronic inflammation observed in fibrosis can be replicated in vitro.

keywords: extracellular matrix, perfusion, bioreactor, immune cells

94238120655

GRAPHENE OXIDE PROMOTES EPITHELIAL MESENCHYMAL TRANSITION IN OVINE AMNIOTIC EPITHELIAL STEM CELLS AFFECTING THEIR IMMUNOMODULATORY PROPERTIES

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Introduction
Regenerative medicine is focusing the attention on immune engineering strategies taking advantage of biomaterials to influence stem cells fate and to stimulate the production of immune modulatory factors to be employed in cells free treatment (1). The Graphene Oxide (GO) was proved to be able to affect stem cells behaviour and to modulate their immune response (2). According to this evidence, the present project was designed to produce secretome with immune modulatory potential by combining GO and/or LPS with Ovine Amniotic Epithelial Stem Cells (oAECs), which exhibited teno-regenerative and high immunomodulatory properties in vitro and in vivo (3), to be used in tendon regenerative medicine.

Methodology
AECs isolated from the amniotic membrane of ovine fetuses (4) were seeded in standard condition (AEC) or with 25µM of progesterone (AEC+P4) (4) or on GO functionalized cover slides (GO). At 70% of confluence, the cells were treated with 1 µg/ml LPS (4) for 1h (AEC+LPS; AEC+P4+LPS, GO+LPS), and incubated for 24h in the Serum Free media. At the end of the experiment, the Epithelial Mesenchymal Transition (EMT) process was assessed within the different tested group by analysing Cytokeratin 8 (CYTO8) and Vimentin (VIM), epithelial and mesenchymal markers respectively, protein expressions and by evaluating the involvement of pSMAD2 and SMAD2/3 pathways. The collected conditioned media (CMs) were used to analyse the profile expression of 40 immunomodulatory cytokines by Inflammation Antibody Array Membrane.

Results
The results demonstrated that GO alone or with LPS (GO+LPS) induces AEC morphology changes shifting the protein patterns expression toward the mesenchymal phenotype, as showed by

negativity to CYTO8 and positivity to VIM. This data suggests the GO accelerated EMT process in AEC and AEC+LPS. In contrast, P4 was able to maintain the epithelial phenotype with higher CYTO8 expression in AEC (AEC+P4) (4) also after inflammatory stimulus (AEC+P4+LPS). Moreover, the upregulation of pSMAD2/SMAD2 proteins ratio was observed in GO group and especially in GO+LPS samples compared to those present in AEC, AEC+LPS, AEC+P4 and AEC+P4+LPS ($p<0.001$) supporting the EMT transition GO-dependent. Furthermore, preliminary inflammatory array assay results on cells CMs suggested that GO was able to modify the cytokines expression profile in cells secretome, by reducing in particular the anti-inflammatory IL10, IL11, IL13 TIMP2 (tissue metalloproteinase inhibitor 2) and CXCL9 (recruiter of leukocytes) chemokine release highly induced in AEC after LPS treatment.

Conclusions
These preliminary data demonstrated that GO accelerates the EMT process in AEC altering their immune response and affecting the release of immune factors. In order to employ these secretomes in regenerative medicine, more experiments are needed to deepen the knowledge of the link between EMT and immunomodulation and to evaluate their biological effects on immune cells.

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keywords: Immune regenerative strategies, AECs, secretome, Graphene Oxide, Tendon regeneration

94238145927

PARTICLE SIZE IN FREE-PACKED GRANULAR SYSTEMS INFLUENCE CELL RESPONSE

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Cell-extracellular communication in granular systems might be explored for tissue engineering and to understand and mimic physiological responses. Particulate systems can be designed as attractive platforms with free movement controlled mainly by intracellular forces and cell migration. We here explore the possibility that the size of the particles composing these systems might have a role in cell response in a sense that a successful long-term cell-particle adhesion might require a minimum traction force for the bond reinforcement. In this work, commercial polystyrene microspheres (type I-collagen coated) are freely assembled and loosely packed as a quasi-3D granular system in a liquid environment. Three size ranges of microspheres (14-20 μm, 38-45 μm, 85-105 μm) were chosen to evaluate the response of human mesenchymal stem cells derived from the adipose tissue (hASC) in these spherical substrates. Cellular characterization was evaluated from 4 hours to a week via metabolic activity, cell adhesion and morphology. Experimental data indicates that objects with increasing diameters (from ~40 μm to ~100 μm) are able to sustain cell adhesion and promote proliferation within seven days of culture. On the other hand, the less explored size comprising 14-20 μm microparticles is more susceptible to cell-mediated mobility, arresting a cell-ECM reinforcement causing early cell detachment. Mechanistic experimental controls through particle sintering allowed to overcome particle mobility and promote cell adhesion in small particles (14-20 μm) as well as increased viability. Weakening cell contractility in larger microspheres (85-105 μm) difficulted the adhesion reinforcement contributing to cell detachment in an otherwise favourable substrate for long-term cell maintenance. Furthermore, an in-silico model addressing pertinent mechanisms of cell attachment to particle beds was developed, corroborating particle free and fixed scenarios. Combining such models with biological assessments could ease the understanding and design of innovative platforms for healthcare-associated problematics.

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keywords: granular system, free-packed, mesenchymal stem cells

20941830804

ELECTROACTIVE MATERIALS GOVERN CELL BEHAVIOR THROUGH THEIR EFFECT ON PROTEIN DEPOSITION

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Smart materials, that react in a controllable and reversible way to external stimuli by varying a specific physical or chemical quantity, show great potential for the development of advanced biomedical strategies, including biosensing, tissue regeneration and repair, immuno- and cancer therapy.1 Among the different types of smart materials, piezoelectric ones, that convert mechanical solicitations into electrical potential variations and vice versa, represent suitable candidates to induce specific cell behaviors through electric cues, mainly by improving biomimicry of the cell microenvironment.2
Although the presence of piezoelectric properties in different tissues is known, their specific influence on cells is still unknown. Mesenchymal stem cells (MSCs) have been found to show increased formation of focal adhesions when cultured on negatively charged substrate.3
Triggering the piezoelectric effect, MSCs show increased differentiation on positively and negatively charged surfaces compared to those cultured on neutral substrates, exposing the importance of combined electrical and mechanical stimulation for bone-regeneration.4,5
One of the main obstacles for translating these materials into clinical practice is the lack of proper understanding of the mechanisms controlling the cellular response to them as well as to the fact that biomaterial-cell interactions are often mediated though proteins, fact that has not been properly addressed in the case of electroactive biomaterials. In fact, the behavior of cells is strongly influenced by conformation of extracellular-matrix proteins,6 and the electrostatic forces of the surfaces to which these proteins adhere, determine their conformation. Thus, we hypothesize that the impact of electrical stimulation on cells can be also tuned by the effect of the physicochemical properties of the biomaterials on the proteins that cover them. However, the influence of electrical stimulation on the material-protein interface remains largely undescribed.
In the present work, we explore the effect of the electric cues on the deposition of extracellular proteins on piezoelectric poly(vinylidene fluoride), PVDF, surfaces with distinct net surface charge, and how these differences affects MSC fate. Using microscopic, spectroscopic, and biochemical techniques, we have uncovered large differences in the deposition dynamics, surface coverage and supramolecular organization of collagen and fibronectin as a function of the electrical charge of the surface to which they adhere. Specifically, positively and negatively charged PVDF surfaces promote proteins adsorption, showing higher amount of protein immobilized on the surface compared to neutral PVDF. Regarding cell response, our semi-automatic analysis of fluorescently-stained MSCs, revealed significant differences not only in MSC spreading and nuclear area, but also in the focal adhesion density.
The presented results allow to regulate the structural features of the deposited extracellular proteins through the control of the surface charge, in order to guide cellular behavior and to obtain specific responses.

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Acknowledgments:
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keywords: smart materials, piezoelectricity, collagen, electroactivity

62825433604

EVALUATION OF TISSUE INTEGRATION AND ANGIOGENESIS OF 3D PRINTED POROUS SCAFFOLDS USING A NON-DESTRUCTIVE MICROCT APPROACH

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Introduction
Among scaffold preparation approaches, biofabrication can provide tridimensional scaffolds with precise and defined architectures that can promote tissue integration and neoangiogenesis after implantation (1). Therefore, the evaluation of tissue ingrowth and vascularization of 3D printed scaffolds is a crucial step in establishing a functional scaffold that could direct the successful tissue regeneration (2). The hen chorioallantoic membrane (CAM) has been proposed as a biorelevant alternative to animal studies for the assessment of implantable scaffolds in vivo. However, current approaches to quantify integration and vascularization, such as histology, lack of spatial resolution. As an alternative, microCT can be helpful to study the extent of tissue integration and vessel architecture within porous implants (3). In this work, we developed a simple and non-destructive method to evaluate tissue ingrowth and neoangiogenesis in 3D printed scaffolds using a CAM model, and further validated in two distinct porous architectures.

Methodology
PLA scaffolds (10 mm in diameter, 4 mm height) with 500 µm pore size designed with/without open lateral porosity were prepared by 3D printing. Sterilized scaffolds were placed on the CAM of fertilized eggs previously incubated for 7 days (Coren, Spain) and incubated at 37 °C for 5 or 7 days. In order to quantify the scaffold integration and vascularization, the CAM tissue surrounding the scaffolds (25 mm in diameter) was fixed in paraformaldehyde, extracted using a scalpel, and immersed in lugol 0.1% for 4 h. Then, samples were washed in PBS, water excess removed and then, scanned at 5 µm voxel resolution using a Skyscan 1272 microCT (Bruker, Belgium). Tissue ingrowth was obtained from the structural changes of structure volume before and after incubation. Finally, the structure and porosity of the reconstructed samples and the new vessels developed within the structure of the scaffolds were analyzed using CTAn software (Bruker, Belgium). Histological analysis was carried out in paraffin-embedded samples after microCT analysis to further quantify new tissue ingrowth and vessel formation and dimensions.

Results
Scaffolds designed with open lateral porosity showed a significant higher integration with the CAM since tissue migrated towards the inner regions of the scaffolds not only from the underneath but also from the side surface of the scaffolds. The incubation with lugol allowed for the precise visualization of vessels and new tissue growing within the scaffolds due to the distinct contrast-enhanced radiopacity of vessels, tissue and scaffold components, as validated by histological and histomorphometrical analysis.

Conclusion
The developed methodology enabled the precise evaluation of tissue integration and vascularization in an embryonic in vivo model. As a non-destructive technique, the developed microCT method is a robust approach for initial in vivo screening of novel porous biomaterials, while complying with the principles of 3Rs.

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keywords: 3D printing, chorioallantoic membrane, CAM, microCT, angiogenesis, tissue integration

S50
One health, one medicine: What Veterinary regenerative medicine can teach us
Room: S3 B
(1 Jul 2022, 11:00 - 12:30)

Conveners: Debbie Guest, Michelle Teunissen, Iris Gerner

83871201986

THE UTILTIY OF EQUINE PLURIPOTENT STEM CELLS FOR THERAPEUTIC USE AND DISEASE MODELLING

Debbie Guest (Royal Veterinary College, Hatfield, United Kingdom)

Introduction
We have derived horse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Pluripotent stem cells undergo unlimited self-renewal and can differentiate into every tissue of the body; properties which can be utilised for therapeutic applications and disease modelling. We have investigated their application in tendon injuries and catastrophic bone fractures, conditions that have major welfare and economic impacts on equine industries. Tendon injuries are a leading cause of retirement in horses taking part in a range of disciplines and catastrophic fracture is the number one reason for euthanasia during Thoroughbred racing.

Adult tendon injuries repair through scar tissue formation which predisposes horses to high rates of re-injury and novel therapies to aid tendon regeneration are required. Equine ESCs turn into tendon cells following their injection into the injured tendon1 and we have established 3D in vitro culture methods2 to define their properties and compare them to adult cells.
The risk of catastrophic fracture is influenced by environmental and genetic factors. A better understanding of the genetic risk factors would enable improved identification and management of high risk horses. Equine iPSCs can be derived from horses with different genetic backgrounds. This has allowed us to establish an in vitro model3 to understand the genetic basis of catastrophic fracture risk.
Methodology
We have established protocols to differentiate ESCs and iPSCs into tendon and bone (osteoblast) cells using both 2D and 3D culture methods.
Global gene expression profiling using RNA sequencing has been used to make comparisons between ESC-derived, adult- and fetal-tenocytes, and between iPSC-osteoblasts derived from horses at high and low genetic risk of fracture.
Other properties of ESC-tenocytes such as cell migration and response to inflammation have also been measured. Molecular biology techniques to understand the impacts of DNA variants associated with fracture are also being used.

Results
We have demonstrated that ESC-tendon cells more closely resemble fetal than adult tenocytes4 but that they have properties that are unique to them, such as resistance to inflammation5 and unpublished data.
iPSC-osteoblasts taken from horses at high and low risk of fracture were found to have 112 differentially expressed genes. Some of which have known roles in bone formation or fracture. Pairing gene expression information with whole genome sequencing data allows us to identify putative causal single nucleotide polymorphism (SNPs) that may be responsible for these differences and we highlight results for COL3A1 that demonstrate the power of this approach.

Conclusions
Pluripotent stem cells from horses not only provide a source of cells for potential therapeutic use, but they can also be used for disease modelling. They therefore hold great promise to allow the future development of novel interventions and therapies.

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keywords: Horse, embryonic stem cells, induced pluripotent stem cells, tendon injury, bone fracture

20941815455

SYNOVIAL MEMBRANE-DERIVED MESENCHYMAL PROGENITOR CELLS FROM OSTEOARTHRITIC JOINTS IN DOGS POSSESS LOWER CHONDROGENIC-, AND HIGHER OSTEOGENIC CAPACITY COMPARED TO NORMAL JOINTS

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Background
Synovial membrane-derived mesenchymal progenitor cells (SM-MPCs) are a promising candidate for the cell-based treatment of osteoarthritis (OA) considering their in vitro and in vivo capacity for cartilage repair. However, the OA environment may adversely impact their regenerative capacity. There are no studies for canine (c)SM-MPCs that compare normal to OA SM-MPCs, even though dogs are considered a relevant animal model for OA. Therefore, this study compared cSM-MPCs from normal and OA synovial membrane tissue to elucidate the effect of the OA environment on the MPC numbers indicated by CD marker profile and colony-forming unit (CFU) capacity, and the impact of the OA niche on tri-lineage differentiation.

Methods
Normal and OA synovial membrane were collected from the knee joints of healthy dogs and dogs with rupture of the cruciate ligaments. The synovium was assessed by histopathological OARSI scoring and by RT-qPCR for inflammation/synovitis-related markers. Presence of cSM-MPCs in the native tissue was further characterized with flow cytometry, RT-qPCR and immunohistochemistry, using the MPC markers; CD90, CD73, CD44, CD271, and CD34. Furthermore, cells isolated upon enzymatic digestion were characterized by CFU capacity, and a population doublings assay. cSM-MPCs were selected based on plastic adherence, expanded to passage 2 and evaluated for the expression of MPC-related surface markers and tri-lineage differentiation capacity.

Results
Synovial tissue collected from the OA joints had a significantly higher OARSI score compared to normal joints, and significantly upregulated inflammation/synovitis markers S100A8/9, IL6, IL8 and CCL2. Both normal and OA synovial membrane contained cells displaying MPC properties, including a fibroblast-like morphology, CFU capacity, and maintained MPC marker expression over time during expansion. However, OA cSM-MPCs were unable to differentiate towards the chondrogenic lineage and had low adipogenic capacity in contrast to normal cSM-MPCs, whereas they possessed a higher osteogenic capacity. Furthermore, the OA synovial membrane contained significantly lower percentages of CD90+, CD44+, CD34+ and CD271+ cells.

Conclusions
The OA environment has adverse effects on the regenerative potential of cSM-MPCs,

corroborated by decreased CFU, population doubling and chondrogenic capacity compared to normal cSM-MPCs. OA cSM-MPCs may be a less optimal candidate for the cell-based treatment of OA than normal cSM-MPCs.

keywords: CD271, CD34, flow cytometry, immunohistochemistry, tri-lineage differentiation

41883615355

THE CROSS-TALK BETWEEN THE SYNOVIAL MEMBRANE AND CARTILAGE IN THE DISTRACTED CANINE KNEE JOINT

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Background
Osteoarthritis (OA) is a common degenerative joint disease, that affects the whole joint. Knee joint distraction (KJD) has been proposed as an alternative joint-preserving treatment strategy for relative young patients with end stage OA. Although there is evidence for the clinical and structural benefits of KJD, the regenerative mechanisms behind KJD remain unclear. This study explores the role of the synovial membrane (SM), an important, but to date limited researched part of the distracted joint, by studying the SM secretome and its effect on chondrocytes.

Methods
Osteoarthritis was bilaterally induced using the groove OA model and allowed to develop for 10 weeks in 12 dogs. Subsequently, KJD was applied unilateral to the right hindlimb for 8 weeks. To determine the role of the SM during KJD, the SM and conditioned medium of the synovial membrane of OA (OA-CM) and OA+KJD joints (KJD-CM) were investigated directly after KJD treatment (n=4), and after 10 weeks of follow-up after KJD treatment (n=8) using RT-qPCR analysis and a canine-specific multiplex ELISA. Furthermore, clinically normal articular chondrocytes (n=3), synovial membrane (n=6) and CM (normal-CM) from healthy dogs were collected for comparison. The effect of OA-CM and KJD-CM on isolated normal articular chondrocytes was investigated using RT-qPCR and Nano luciferase response element reporter assays for targeted signalling analysis of pathways involved in anabolic, catabolic and inflammatory processes during OA.

Results
In the synovial membrane and conditioned media the expression of pro-inflammatory cytokines (CCL2, IL-6, IL-7, IL-15, and IL-18) was increased directly after KJD compared to the normal SM. After 10 weeks of follow-up, these cytokines were still elevated, except for IL-6, compared to directly after KJD. Directly after KJD, the OA and the KJD-CM had a clear catabolic effect on articular chondrocytes, as established by decreased expression of the cartilage matrix genes ACAN and COL2A1 and increased expression of the catabolic genes MMP13 and ADAMTS5. After 10 weeks of follow-up, this effect shifted towards a less catabolic response. The reporter assays showed an upregulation of CRE and SIE signalling after stimulation with OA-CM and KJD-CM compared to normal-CM. SRE and SRF signalling was downregulated in the presence of the KJD-CM at follow-up compared to the KJD-CM from directly after KJD.

Conclusion
OA and KJD result in inflammation of the SM, characterized by increased levels of pro-inflammatory cytokines and chemokines. The SM secretome directly influences cartilage

matrix metabolism and cellular response, corresponding with the catabolic environment in the cartilage that is found directly after KJD. After follow-up the catabolic influence of the SM decreases. Therefore, the SM may contribute to the effect that joint distraction exerts on the OA joint. However, as the effect is small, in order to gain further insights, the sample size should be increased. Furthermore, more cartilage protective pathways should be investigated to investigate the role of the SM in the regenerative mechanisms behind joint distraction.

keywords: Knee joint distraction, conditioned medium, cytokines, RT-qPCR, response element reporter assays

83767226284

SHEEP CELLS AS A SUITABLE IN VITRO TOOL TO EVALUATE INTERVERTEBRAL DISC BIOTHERAPIES

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Introduction
Intervertebral disc (IVD) degeneration is among the leading cause of low back pain, disability and morbidity worldwide. As the world population ages and only symptomatic treatment exist, IVD regeneration is a major public health challenge for the upcoming years. Human IVD cells are difficult to obtain, especially healthy ones, and murine IVDs present numerous differences with human ones (size, mechanical loading, presence of notochordal cells). The sheep spine exhibits biological and biomechanical similarities with the human one and is thus recognized as an appropriate model for translational applications1. With the goal of reducing our reliance on animal models, and for economic, regulatory, and ethical reasons, we have set up an in vitro platform based on sheep annulus fibrosus (AF) and nucleus pulposus (NP) cells to evaluate cell and extracellular vesicles (EVs) therapies.

Methodology
Cells from both the AF and NP were isolated from the IVDs of five young sheep (≈ 3 months old). Their metabolic activity and gene expression were evaluated by CCK-8 assay and RT-qPCR. To simulate a degenerative IVD microenvironment, disc cells were treated with either recombinant sheep IL-1β (10 ng/mL) or H2O2 (500 μM) or maintained in culture for over 10 passages. EVs from human adipose-derived mesenchymal stromal cells (hASCs) were produced in a turbulent flow as previously described2 and their effect on degenerative-like NP and AF cells was evaluated.
In a separate experiment, hASCs cells were co-cultured with NP or AF cells, in direct contact or transwells. The use of two species allowed us to analyze RNA expression from disc cells in direct

co-culture with hASCs by using sheep-specific primers.

Results
Prolonged culture and treatments with IL-1β or H2O2 led to a significant overexpression of inflammatory cytokines (IL6, CXCL8), matrix metalloproteinases (MMP1, MMP2, MMP3, or MMP13 depending on the treatment), and downregulation of key components of the extracellular matrix (COL1A1 & COL2A1) at the transcriptional level. While EVs consistently increased basal metabolic activity of both AF and NP cells at early and late passages, they had little effect on gene expression. On the other hand, direct cocultures with human ASCs profoundly affected disc cells' transcriptional profile. Notably, both types of cocultures led to a drastic downregulation of CXCL8 in disc cells, reduced by over 60% in indirect coculture and even undetectable in direct coculture, while we observed an upregulation of COL1A1 but also MMP1. Surprisingly, IL6 expression showed a slight increase with hASCs on transwells but a sharp decrease by over 80% when hASCs were in direct contact.

Conclusion
We demonstrated that healthy sheep cells expressed markers of degeneration after IL-1β and H2O2 treatment, or after numerous passages in culture. They showed biological responses to hASCs and, to a lesser extent, to hASC-derived extracellular vesicles. These results confirm the suitability of sheep disc cells to model IVD degeneration in vitro and assess biotherapies.

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keywords: intervertebral disc, regenerative therapy, sheep, mesenchymal stromal cell, extracellular vesicles

83767231959

PHENOTYPIC CHARACTERIZATION OF ADIPOSE-DERIVED MSC BASED ON THEIR PHOSPHOLIPID PROFILES

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The phenotypic characterization of multipotent mesenchymal stromal cells (MSC) still suffers from deficits and the resulting heterogeneity of MSC used in different preclinical and clinical studies hamper the translational success. In search for novel MSC characterization approaches to complement the traditional trilineage differentiation and immunophenotyping assays reliably across species and culture conditions, this study explored the applicability of lipid phenotyping for MSC characterization and discrimination. Human peripheral blood mononuclear cells (PBMC), human fibroblasts, and human and equine adipose-derived MSC were used to compare different mesodermal cell types and MSC from different species. For MSC, cells cultured in different conditions, including medium supplementation with either fetal bovine serum or platelet lysate as well as culture on collagen-coated dishes, were additionally investigated. After cell harvest, lipids were extracted by chloroform/methanol according to Bligh and Dyer. The lipid profiles were analysed by an untargeted approach using liquid chromatography coupled to mass spectrometry (LC-MS) with a reversed phase column and an ion trap mass spectrometer. In all samples, phospholipids and sphingomyelins were found, while other lipids were not detected with the current approach. The phospholipids included different species of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) in all cell types, whereas phosphatidylglycerol (PG) species were only present in MSC. MSC from both species showed a higher phospholipid species diversity than PBMC and fibroblasts. Few differences were found between MSC from different culture conditions, except that human MSC cultured with platelet lysate exhibited a unique phenotype in that they exclusively featured PE O-40:4, PG 38:6 and PG 40:6. In search for specific and inclusive candidate MSC lipid markers, we identified PE O-36:3 and PG 40:7 as potentially suitable markers across culture conditions, at which PE O-36:3 might even be used across species. On that basis, phospholipid phenotyping is a highly promising approach for MSC characterization, which might condone some heterogeneity within the MSC while still achieving a clear discrimination even from fibroblasts. Particularly the presence or absence of PG might emerge as a dinstinctive criterion for future MSC characterization.

This study has recently been published under the creative commons license in Front Cell Dev Biol. (2021 Dec 1;9:784405; doi: 10.3389/fcell.2021.784405).

keywords: MSC, phenotyping, phospholipids

31412730807

HOW DO INFLAMMATION, DIFFERENTIATION, AND MHC COMPATIBILITY AFFECT THE IMMUNOGENICITY AND IMMUNOMODULATORY POTENTIAL OF EQUINE MESENCHYMAL STEM CELLS (MSCS)?

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Introduction

Immunomodulation and immunogenicity of mesenchymal stem cells (MSCs) may influence their efficacy and safety, thus being key for their therapeutic use. The immune regulatory mechanisms of MSCs depend mostly on the secretion of different mediators which are not only important for the MSC therapeutic mechanisms but also to facilitate their escape from immune recognition when administered allogeneically. Actually, a highly relevant paradigm change is that MSCs are not truly immune-privileged but immune-evasive, and thus, their recognition and elimination by the immune system in the allogeneic setting should be considered. Since the horse is highly valuable as both patient and translational model, further knowledge on equine MSC immune properties is required. This study analyzed how inflammation, chondrogenic differentiation and compatibility for the major histocompatibility complex (MHC) influence the MSC immunomodulatory-immunogenicity balance by studying the changes elicited in vitro by equine MSCs on relevant lymphocyte subpopulations.

Methodology

Equine MSCs in basal conditions, pro-inflammatory primed (MSC-primed) or chondrogenically differentiated (MSC-chondro) were co-cultured with either autologous or allogeneic MHC-matched/mismatched lymphocytes. Two types of co-cultures were used: immunosuppressive assays to study MSC immunomodulatory potential, and one-way modified mixed leukocyte reactions (MLRs) to assess MSC immunogenicity. Lymphocytes were stained with

carboxyfluorescein succinimidyl ester (CFSE) and with a panel of antibodies to study changes in the frequency and proliferation of T cell subsets (cytotoxic, helper and regulatory) and B cells by flow cytometry.

Results
Overall, MSC-primed were superior suppressing the proliferation of lymphocytes, followed by MSC-chondro and MSC-naïve. The proliferation of CD3+ T cells was reduced in the presence of all types of MSCs and for all the combinations (autologous and allogeneic MHC-matched and mismatched). When looking at specific lymphocyte subsets, MSC-primed showed higher regulatory potential of the proliferation of cytotoxic and helper T cells and B cells, while inducing T reg cells in the one-way MLRs. However, MHC-mismatched MSC-primed can also elicit a proliferative response in lymphocytes likely due to increased MHC expression. Interestingly, equine MSC-chondro maintained their regulatory ability and did not increase their immunogenicity, but showed less capacity than MSC-primed to induce regulatory T cells and further stimulated B cell proliferation.

Conclusions
Priming MSCs with proinflammatory cytokines activates their regulatory potential. However, inflammation can also increase the immune recognition of these cells through induction of MHC expression, thus making the allogeneic MHC-mismatched MSC-primed more likely to be targeted by the immune system. Importantly, equine MSCs do not lose their regulatory ability neither increase their immunogenicity after chondrogenic differentiation, but have reduced capacity to stimulate Treg cells and can stimulate the proliferation of B cells.
Even though lymphocyte proliferation assays are important tools to assess both the immunomodulatory and immune evasive properties of MSCs, subsequent in vivo studies are needed to elucidate the complex interactions between MSCs and the recipient immune system, which is critical to develop safe and effective therapies.

keywords: mesenchymal stem cells, horse, immune response, MHC-haplotype, flow cytometry

52354523526

EVS IN EQUINE REGENERATIVE MEDICINE – CHALLENGES AND POTENTIAL THERAPEUTIC IMPLICATIONS

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Mesenchymal stem/stromal cell (MSC) derived EVs have shown an equivalent therapeutic potential to their donor cells, making them a promising tool for regenerative medicine. However, non-standardised EV isolation methods and the limited availability of cross-reacting markers for most animal species restrict comparability and standardisation of animal experiments.
We therefore performed a study to establish an EV isolation and characterisation protocol for equine MSC-derived EVs and define minimal criteria based on the ISEV criteria, which will facilitate reproducibility and comparison of equine derived EVs between studies in the future. Furthermore, we studied the therapeutic effect of equine bone marrow MSC (bmMSC) derived EVs on inflamed horse tenocytes in vitro and evaluated the influence of inflammatory preconditioning (pc) of the EV donor MSC on the content and biological activities of their EVs.
Equine bmMSC dericed EVs from 3 donors were isolated using two different techniques, size exclusion chromatography (SEC) and ultracentrifugation technique (UC). To establish minimal criteria for equine EVs, we validated markers (CD63 and CD9) for Western blot analysis and tested a combination of methods including techniques that do not require antibodies, such as Nanoparticle Tracking Analysis (NTA) and trans electron microscopy (TEM), with Fluorescence-triggered flow cytometry (FT-FC).
To evaluate their therapeutic effect, EVs were obtained from supernatants of bmMSCs preconditioned in serum free media with (pcMSC) or without (npcMSC) addition of 10ng/ml IL1β and 10ng/ml TNFα. Chemically inflamed (10ng/ml IL1β and 10ng/ml TNFα) tenocytes (400.000 cells) were treated with 1ml of medium containing 1x10^9 autologous pcMSC or npcMSC derived EVs. Untreated chemically inflamed tenocytes and healthy tenocytes served as control groups. RNA-Sequencing (RNA-Seq) of EVs and tenocytes was performed. Adjusted p-value was set at 0.25.

The isolated particles stained positive for CD9 and CD63 as demonstrated by Western blot and for CD81 using FT-FC. FT-FC, NTA and TEM confirmed the EV-appropriate size range (between 30 and 150 nm) and TEM the presence of the characteristic lipid bilayers surrounding EVs.
Application of pcMSCs as well as npcMSC derived EVs significantly reduced expression of inflammation markers like CXCL6 (-1.4 and - 1.6 logFC), CSF3 (-3.3 and - 4.1 logFC), CXCL8 (-2.2 and - 2.2 logFC), CXCL6 (-1.4 and -1.6 logFC), TNFAIP6 (-1.4 and - 1.4 logFC) in treated tenocytes as compared to untreated controls. miRNA-Seq of the EVs showed significant down-regulation of miR-146a (-0.6 and -0.7 log2FC) which has been shown to play an important role in the negative regulation of inflammation in rheumatoid arthritis.
The obtained data allows to suggest minimal criteria for the standardized isolation and characterization of equine MSC derived EVs. The results further indicate that the cargo of pcMSC-EVs and npcMSC-EVs may reduce tenocyte inflammation and may have an immunomodulatory effect on the recipient cells which is independent of pre-conditioning of the EV donor cells.

keywords: extracellular vesicles, equine, characterisation, tendinitis treatment

31412717204

INDUCTION OF THE SENESCENCE PHENOTYPE IN EQUINE TENDON DERIVED CELLS BY DEXAMETHASONE

Neda Heidari (Royal Veterinary College, Hatfield, United Kingdom), Roger Smith (Royal Veterinary College, Hatfield, United Kingdom), Richard Faragher (University of Brighton, Brighton, United Kingdom), Jayesh Dudhia (Royal Veterinary College, Hatfield, United Kingdom)

Introduction
Tendon injuries in humans are a major healthcare concern but they also occur spontaneously in other species including horses. The superficial digital flexor tendon (SDFT) of the horse is a highly relevant model for studying tendinopathies because it is a functional homologue of the Achilles tendon with similarities in aetiopathogenesis and associated risk factors (1,2). Previous studies with cyclical loading of tendon explants have revealed that injured SDFTs produced high levels of pro-inflammatory cytokines and 10-1000-fold overexpression of matrix metalloproteinases with impaired resolving mechanisms, especially in older horses (3). In combination, these markers are hallmarks of senescent cells. Senescent cells are generated by many pathways (both cell division dependent and independent) all of which produce viable cells that (a) overproduce pro-inflammatory cytokines collectively known as the Senescence Associated Secretory Phenotype (SASP) and (b) actively remodel matrix, all of which are implicated in age-related degenerative changes that predispose to tendinopathies. Corticosteroids frequently used clinically to treat tendinopathies initially block the inflammatory component of tenocyte senescence but also may cause therapy-induced senescence and interfere with tendon repair, contributing to the high re-injury rate (4). The aim of this study was therefore to use an in vitro model to investigate the effects of dexamethasone on senescence.

Methodology
Tendon derived cells (TDCs), isolated from the superficial digital flexor tendon (SDFT) of adult horses (11 years old), were cultured in DMEM (supplemented with 10% fetal bovine serum and 1% P/S) in a humidified incubator with 5% CO2 at 37°C. TDCs were treated with 1 and 10µM dexamethasone for 48 hours and then cultured in medium without dexamethasone for a further 24 and 72h. The effect of dexamethasone on cell viability was measured by the MTT assay at 24 h. Senescence was confirmed in treated cells by cytochemical analysis with EdU (Click-iT Plus EdU Cell Proliferation Kit, Fisher) and Ki-67 (Rabbit monoclonal to Ki67, Abcam).

Results
The viability of TDCs was not affected by 1 and 10 µM doses of dexamethasone treatment. However, dexamethasone at both concentrations inhibited cell proliferation and induced cell cycle arrest. Senescence in TDCs was confirmed by reduced expression of proliferative-associated protein (Ki-67) and reduced DNA synthesis (EdU incorporation). Exposure to dexamethasone at both doses for 48 hours rendered more than 50% of the tenocytes senescent.

Conclusion
Dexamethasone at clinically relevant doses induced growth arrest in equine tenocyte cells. These data provide a mechanistic explanation for potential adverse effects of using corticosteroids for the treatment of tendinopathies. The model will enable investigations of novel candidate molecules that can slow or stop the degenerative process by inhibiting

senescence in vitro, which could be used to improve the clinical benefits of corticosteroids via its anti-inflammatory effects.

References

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keywords: Tendinopathy, Senescence, Glucocorticoids, SASP

S51+S29
Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine + Engineered viscoelasticity in cell and tissue engineering
Room: S4 A
(30 Jun 2022, 15:30 - 17:00)

Conveners: João Mano, Jacek K. Wychowaniec, Aline F. Miller

20967804648

DYNAMIC HYDROGEL DESIGN FOR SPATIOTEMPORAL CONTROL OF MORPHOGENESIS

Nicolas Broguiere (EPFL, Lausanne, Switzerland), Matthias Lütolf (EPFL, Lausanne, Switzerland), Marcy Zenobi-Wong (ETH, Zürich, Switzerland), Antonius Chrisnandy (EPFL, Lausanne, Switzerland)

3D culture and organoid technologies have been developing rapidly in the last decade, and already found widespread applications in biology and medicine. While cells, and stem cells in particular, have tremendous self-organization potential, most applications benefit from further engineering of the cellular microenvironment in order to guide the morphogenesis.

We will describe several recently developed technologies that exploit dynamic physical processes to support or guide morphogenesis in defined 3D cultures. In one instance, we formed hydrogels with reversible, dynamic bonds, in order to impart polyethylene glycol (PEG) hydrogels with stress relaxation and self-healing properties. The resulting hydrogels could support intestinal stem cell expansion as well as differentiation to budding organoids in a single defined hydrogel. In another instance, we exploited a dynamic aqueous-aqueous phase separation triggered by PEG cross-linking in order to gain control over the pore structure of the PEG gels. The method is simple, cost-effective, compatible with injection and cell encapsulation, yields clear hydrogels, and easily tuned to obtain pore sizes of any relevant size, from less than 1 to more than 100 micrometers. These hydrogels proved optimal to support the formation of functional 3D neural networks in a defined environment. Finally, we will show how morphogenesis can further be guided with dynamic patterning of morphogens / growth factors, using a recently developed two-photon patterning method, compatible with sensitive biomolecules, live cells, and both natural and synthetic extracellular matrices.

We believe such tailored microenvironments, exploiting dynamic processes to guide morphogenesis, will be key to the development of 3D engineered tissues with a higher degree of cellular organization.

keywords: Dynamic hydrogels, two-photon patterning, organoids, brain, intestine

94355106246

HYDROGELS THAT TALK TO CELLS WHEN LIGHTED

Aránzazu Del Campo (INM - Leibniz Institute for New Materials, Saarbruecken, Germany)

Growth factor (GF) based therapies in regenerative medicine are limited by the high cost, fast degradation kinetics, and lack of specificity as consequence of the multiple functions of GF in the cell. One common GF therapeutic scenario is the administration of VEGF to support vascularization during tissue regeneration. The therapeutic window for VEGF treatment is narrow: low doses are safe but not sufficient to yield a therapeutic benefit, and slightly higher doses lead to the growth of angioma-like vascular structures. Moreover, angiogenesis in vivo occurs by sustained angiogenic stimulus over a month to achieve stable vessels, and the outcome of the process is highly dependent on the spatiotemporal distribution of the proangiogenic signal. In this talk different approaches to control the presentation of angiogenic signals in biomaterials for tissue regeneration using phototriggers will be presented.

keywords: growth factor, regenerative medicine, living therapeutic materials, VEGF, angiogenesis

52354546605

WET-SPUN CORE-SHELL HYDROGEL FIBERS FOR MICROVASCULAR TISSUE ENGINEERING

Alessia Paradiso (Warsaw University of Technology, Warsaw, Poland), Marina Volpi (Warsaw University of Technology, Warsaw, Poland), Joanna Idaszek (Warsaw University of Technology, Warsaw, Poland), Diana Guerrero-Martinez (Warsaw University of Technology, Warsaw, Poland), Maciej Łojkowski (Warsaw University of Technology, Warsaw, Poland), Marco Costantini (Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland), Wojciech Święszkowski (Warsaw University of Technology, Warsaw, Poland)

Introduction:
Recently, tissue engineering still lacks thorough vasculature, which represents a major drawback in developing physiologically relevant tissue constructs. Among others, the design and the biofabrication of blood vessels at the microscale remain challenging, due to their role in nutrient and oxygen exchange, but also waste removal. Parallelly, fiber-based biofabrication techniques such as 3D-(bio)printing are considered time-consuming approaches in the field of microvascular tissue engineering. In this work, vessel-like 3D core-shell bundles have been rapidly fabricated using a novel wet-spinning system, allowing for the collection of cell-laden hydrogel-based fibers onto a rotating drum to reproduce the native architecture of the microvascular network.

Methodology
Therefore, two different hydrogel formulations were optimized. First, a fibrinogen- and an alginate-based solutions were studied in terms of material characterization. Rheological measurements on the pre-polymer solutions and swelling test on the fibrous scaffolds were carried out to investigate the behavior of these biomaterials. Then, SEM analysis was assessed to evaluate the microstructure of the core-shell yarns. Afterwards, the fibrinogen-based formulation loaded with a co-culture of human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs), and the alginate biomaterial ink were simultaneously extruded from a microfluidic co-axial nozzle immersed in a CaCl2 coagulation bath to produce tissue-specific core and supporting tubular shell structures, respectively. Both motor speed and flow rates were adjusted and tuned to create fibers with a diameter dimension of around 300 μm. Upon instantaneous gelation of alginate, wet-spun fibers were collected to form densely packed fascicles. To further stabilize the bundles, a secondary crosslinking was performed by immersing the yarns in a thrombin solution to induce the enzymatic polymerization of the core. Thus, the engineered constructs were incubated at cell culture conditions for up to 21 days. The metabolic activity of encapsulated cells was evaluated by means of proliferation assay. Subsequently, cell-laden scaffolds were investigated in terms of morphological characterization. Finally, immunocytochemistry will be performed to prove the formation of vessel-like structures.

Results
The material characterization of the proposed formulations exhibited a Newtonian-like behavior, proving the suitability of non-shear thinning hydrogel-based bioinks for wet-spinning. Tissue-specific wet-spun core-shell fibers supported cell adhesion, migration, and alignment over the culture time, generating packed 3D cell-laden constructs that may recapitulate the microvascular network. The proliferation assay confirmed consistent metabolic activity during the cell-culture period. In addition, scaffolds would likely reveal their endothelialization role, highlighting the potential of the two proposed bioinks in the frame of microvascular tissue engineering.

Conclusions
In conclusion, wet-spun fibers were produced from a novel co-axial needle and collected by using functionalized hydrogels, thus validating the system for the (bio)fabrication of Newtonian-like hydrogel-based constructs. Herein, encapsulated MSC-HUVEC migrated within the cell-laden hydrogel core towards the wall of the alginate shell, thus aligning along the direction of the microfibers axis to form a cellular layer. This study aims to highlight a new model to promote microvascular networks. The proposed wet-spinning platform can be considered as a potential alternative to 3D-(bio)printed engineered microvascularized constructs.

Acknowledgments:
The study was supported by the National Center of Research and Developments (grant. No. POLTUR4/BIOCANCER/3/2021)

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keywords: hydrogel fibers, wet-spinning, microvascularization, microfluidics

31412746655

MICROFLUIDIC SPINNING OF HYDROGEL-BASED CORE-SHELL MICROFIBERS FOR THE FABRICATION OF MYOTENDINOUS TISSUE-LIKE CONSTRUCTS

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Myotendinous junction disfunctions due to degenerative musculoskeletal diseases or injuries resulting from strenuous physical activities are still considered an ongoing issue in the field of musculoskeletal tissue engineering. Indeed, the main challenge of biofabrication strategies relies on the development of methods enabling the generation of artificial bio-constructs that can replicate the complexity of the muscle-tendon tissue interface. In this work, a core-shell Y-shaped microfluidic chip has been developed to alternatively deliver two different hydrogel-based bioinks that enable the mimicking of the tendon and the muscle tissue, respectively. As a result, core-shell microfibers are extruded and collected on a rotating drum to form heterogenous hydrogel yarns. In order to fabricate a myotendinous-like construct, the time-switching between the two bioinks, the drum rotational speed, and the core and shell flow rate, have been optimized. Once flow rates have been selected, different values of drum rotational speeds (i.e., 20, 30, 40, 50 and 60 rpm) have been tested to evaluate the overall effect on both the microfiber diameter and the core dimension. Moreover, NIH 3T3 fibroblasts and C2C12 myoblasts, used to mimic the tendon and muscle side respectively, were encapsulated in the core bioink and spun at the selected rotational speed values. As a result, the effect of the increasing velocity induced a decreasing of the microfiber and core diameter along with the shell thickness. Furthermore, rotational speeds up to 40 rpm showed high viability and cell alignment along the fiber direction for both NIH 3T3 fibroblasts and C2C12 myoblasts. On the other hand, rotational speeds of 20 rpm and 30 rpm induced low cell alignment and spreading along with a low cell viability, due to the increasing of shell diameter that prevents the oxygen and nutrient exchange. In addition, it was observed that rotational speeds up to 40 rpm were related with higher switching-time that create flow rate turbulences and a reduced ratio of muscle/tendon tissue-only section compared to the tissue interface one. Hence, 40 rpm has been selected as optimal rotational speed velocity for the fabrication of myotendinous-like constructs. Cell-laden heterogenous scaffolds showed high degree of compartmentalization and enabled the recreation of the tissue-specific biological heterogeneity. Moreover, C2C12/NIH 3T3-laden constructs showed high cell proliferation up to 14 days of culture. Finally, immunochemistry analysis will be performed in order to investigate myosin heavy chain as well as collagen I and III expression at the muscle and tendon side, respectively. Thus, such biofabrication method could be validated for the generation of a biomimetic heterogenous scaffold that can recapitulate the biological complexity of the muscle tendon unit.

Acknowledgments:
The study was supported by the National Centre for Research and Developments in the framework of the project "Advanced Biomaterials and Biofabrication Methods for Engineering of the Myotendinous Junctions" (grant No. PL-TW/VI/3/2019)

keywords: skeletal tissue muscle, microfluidic spinning, hydrogels fibers

31412704686

4D BIOPRINTING OF SELF-BENDING SCAFFOLDS FOR ARTICULAR CARTILAGE TISSUE ENGINEERING APPLICATIONS

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Introduction:
Articular cartilage (AC) defects remain a significant clinical challenge[1]. This is partially due to the challenging nature of recapitulating the complex layered structure observed in the naturally curved AC tissue. While three-dimensional (3D) bioprinting appeared as a promising Tissue Engineering (TE) approach, it has serious limitations in the fabrication of curved constructs[2]. This has motivated the development of four-dimensional (4D) bioprinting as the next generation of biofabrication technologies, combining 3D-bioprinting with time-dependent shape transformation, and introducing time as the fourth dimension[3]. 4D-bioprinting allows for the fabrication of self-bending scaffolds and shape-transforming constructs. In this study, we report an advanced 4D-biofabrication method based on the differential swelling of a multi-material smart bioink.

Methods:
Two biomaterial ink formulations with different swelling properties were selected: tyramine-functionalized hyaluronan (HAT, high-swelling) and alginate with HAT (AHAT, low-swelling). Firstly, the inks were characterized with an MCR-501 rheometer (AntonPaar) to measure their storage/elastic modulus, loss/viscous modulus, shear-thinning, and viscosity. BioX-bioprinter (Cellink) was used to fabricate a bilayered scaffold. The bottom zone was made of HAT and the top zone of AHAT. After printing, the bilayered scaffold was crosslinked in 200 mM CaCl2, and then submerged in saline or DMEM medium. Finally, human bone-marrow derived cells (hMSC) were incorporated into AHAT (top zone) before 4D-bioprinting the bilayered scaffolds. The scaffolds, cultured in chondrogenic medium for 28 days, were analyzed by live/dead and histology.

Results:
Rheological characterization demonstrated that both HAT and AHAT inks had i) similar elastic, gel-like behaviors, as their elastic modulus was 8x higher than their viscous modulus; ii) shear-

thinning behavior, and iii) relatively fast recovery reaching 100% and 65% (respectively) of the storage modulus. After 3D printing, AHAT showed a higher compression modulus than HAT (6.7 vs. 2.1 kPa). Upon 24 h submersion in saline HAT absorbed 2x more liquid than AHAT.
The inks were 3D printed into a bilayer. After time (4D), the differential swelling between the two zones led to the scaffold's self-bending behavior. Different scaffold designs were used to characterize the degree of curvature. The live/dead results demonstrated high cell-viability in the 4D-bioprinted scaffolds. After 28 days, the curvature was still evident, with no delamination observed, and histology suggested an increase in sGAG production.

Discussion and conclusion:
A proof of concept of the recently emerged technology of 4D-bioprinting with a specific application for articular cartilage tissue engineering was achieved. We fabricated smart cell-laden scaffolds with self-bending properties for the design of curved structures mimicking the native AC tissue architecture in specific regions. This approach allowed for the fabrication of a curved bilayer made from two biocompatible and commonly used hydrogel-based materials in TE. Further studies should focus on increasing the mechanical properties of the scaffold, as well as improving the tissue formation through incorporation of tissue-specific biological cues.

Acknowledgments:
Dutch Medical Delta (RegMed4D) and TUD-EMC Convergence Initiative Health & Technology.

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keywords: 4D Bioprinting, Smart Bioinks, Biofabrication, Articular Cartilage

31412715309

CLICKABLE DYNAMIC BIOINKS

Pierre Tournier (Nantes University, Nantes, France), Nathan Lagneau (Nantes University, Nantes, France), Boris Halgand (Nantes University, Nantes, France), François Loll (Nantes University, Nantes, France), Jérôme Guicheux (Nantes University, Nantes, France), Catherine Le Visage (Nantes University, Nantes, France), Vianney Delplace (Nantes University, Nantes, France)

Introduction
Bioprinting is a booming and promising technology to create tissue models, with numerous applications in tissue engineering and regenerative medicine. However, the biomaterials commonly used for bioprinting involve non-physiological stimuli (e.g., sudden changes in temperature, pH, ionic forces) and lack tunability post-printing. These biomaterials are therefore still far from recapitulating the physicochemical and biological characteristics required to create relevant in vitro tissue models. To date, a biologically relevant, ultra-tunable, fast- and easy-to-use bioink platform remains to be invented.

Methodology
We envisioned to create a novel bioink platform with tunable composition and mechanical properties post-printing. To succeed, we hypothesized that a dynamic covalent hydrogel, which can flow, can be modified with a reactive moiety so that mechanical and biochemical properties can be adjusted after printing upon the simple addition of a complementary reactive molecule to the culture medium. Using hyaluronic acid (HA) as a natural polymer of interest, boronate ester crosslinking was investigated for the design of the platform dynamic covalent bioink. Regarding the secondary chemical reaction, we used a « click » reaction, namely the strain-promoted azide-alkyne cycloaddition (SPAAC), able to meet rigorous criteria (i.e., physiological conditions of pH and temperature, no byproducts, no purification, bioorthogonality) and allow post-printing modifications in the presence of cells. By combining these two chemical tools, we successfully created what we called « clickable dynamic bioinks ». We then investigated the feasibility of various post-printing modifications (e.g., stiffening, peptide addition) to drive cell fate and build biologically relevant tissue models.

Results
We demonstrated for the first time that boronate ester crosslinking can be used for the design of printable hydrogels, with non-swelling/non-shrinking, shear-thinning and self-healing properties, tunable viscoelasticity (G’ of 200 to 2500 Pa, at 1 Hz), and in vitro stability over months. We showed that these hydrogels are cytocompatible (>90% viable cells) with various primary human cell types (e.g., MSCs, chondrocytes), and that they can prevent cell sedimentation in a cartridge, circumventing what is a common issue in bioprinting. These new bioinks allowed us to design constructs of various shapes and volumes (tested up to 10 layers). The 3D bioprinted constructs immersed in culture medium can be tuned by simply adding to the medium the SPAAC-modified molecule of interest, which diffuses in the constructs and react with the dynamic network. We showed that the composition of a bioprinted construct can be tuned by adding chondroitin sulfate, low molecular weight HA, gelatin or an adhesive peptide (RGD). This technique also allowed us to increase the rigidity of a construct (G’ increased from 200 to 1200 Pa) or control cell adhesion. Of major value, we demonstrated that these post-printing modifications can be controlled in time and space.

Conclusions

We showed that clickable dynamic bioinks constitute a simple and versatile platform for bioprinting. It carries the hope of easy, fast and cost-effective access to any kind of tissue with adaptable composition and architecture, paving the way to biologically relevant 4D bioprinting, with virtually unlimited tissue engineering applications.

keywords: Hyaluronate, clickable dynamic bioink, 3D bioprinting, cell fate tunability, tissue model

73296334968

GLYCOPEPTIDE-BASED SUPRAMOLECULAR HYDROGELS INDUCE DIFFERENTIATION OF STEM CELLS INTO NEURAL LINEAGES

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Introduction
Short peptide amphiphiles have been widely reported as building blocks of supramolecular hydrogels for biomedical applications1,2, as they can copycat bioactive protein sequences. However, in the extracellular matrix (ECM), proteins are usually present as glycoproteins with different roles, e.g., storage depots of proteins and co-receptors. In this context, the use of self-assembling glycopeptide amphiphiles is gaining an increasing interest3 due to their ability to form supramolecular structures that mimic the ECM. In addition, the fact that they are maintained by non-covalent interactions (e.g., p-p or CH-p stacking) makes them inherently stimuli-responsive and dynamic systems.2 Here, we synthesised a short glycopeptide amphiphile, i.e., Fmoc-diphenylalanine-glucosamine-6-sulfate (Fmoc-FF-GlcN6S), and evaluated its ability to promote neural regeneration.

Methodology
Fmoc-FF-GlcN6S was synthesised by coupling Fmoc-FF and GlcN6S using DCC-NHS chemistry. Two methodologies were used to prepare the Fmoc-FF-GlcN6S hydrogels: 1) temperature switch (T method) – heating at 90 °C to dissolve the amphiphile followed by cooling to room temperature; and 2) solvent-switch (S method) – dissolving the amphiphile in DMSO followed by its dilution into water. The mechanical properties of the generated hydrogels were assessed by rheology, and their supramolecular structure (i.e., molecular packing/interactions and nanofiber morphology) was evaluated using CD, FTIR and AFM. The effect of the generated hydrogels on human adipose-derived stem cell (hASC) behaviour was evaluated by live/dead analysis, immunostaining and qPCR.

Results
Both methods formed hydrogels composed by entangled nanofibers. The stiffness of the hydrogels was influenced by the preparation method: gels generated by the T method were stiffer than the ones formed by the S method, G′ (T) = 2.4kPa > G′ (S) = 0.5kPa. Under both methods, the morphology of the nanofibers was similar (AFM). Interestingly, CD and FTIR analyses demonstrated that peptide glycosylation altered the secondary structure of the nanofibers from β-sheets (for Fmoc-FF) to α-helixes (for Fmoc-FF-GlcN6S).
hASCs cultured on Fmoc-FF-GlcN6S hydrogels showed distinct behaviour dependant on the preparation method: hASCs spread throughout the surface of the hydrogels prepared by the

T method, while cell clusters were observed for hydrogels generated by the S method. qPCR and immunostaining showed that hASCs seeded on both types of hydrogels (i.e., T/S methods) overexpressed GFAP and Nestin on day three and MAP2 and βIII-tubulin on day nine of cell culture.

Conclusions
We demonstrate that Fmoc-FF-GlcN6S is able to self-assemble into biofunctional hydrogels, whose stiffness can be tuned altering the preparation method (i.e., S/T), as well as the concentration of the amphiphile. The Fmoc-FF-GlcN6S hydrogels induce hASC differentiation into neural lineages, being a good indication of their suitability for neural regeneration. In this context, the T method is a more adequate alternative as it does not use organic solvents and the gelation conditions (e.g., gelation timeframe) allows the encapsulation of cells.

Acknowledgements
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keywords: glycopeptide, supramolecular, self-assembly, neural differentiation

S52
Perspectives For Future Innovation in Tendon repair (P4 FIT)
Room: S3 B
(30 Jun 2022, 11:00 - 12:30)

Conveners: Giovanna Della Porta; Nicholas Forsyth

83871202586

ADVANCES IN BIOACTIVE MATERIALS FOR TENDON REPAIR

Aldo Boccaccini (University of Erlangen-Nuremberg, Erlangen, Germany), Liliana Liverani (University of Erlangen-Nuremberg, Erlangen, Germany)

In this presentation the latest advances in the field of biomaterials for tendon repair will be presented. Current technologies put forward for engineering tendon tissue will be discussed. The focus of the presentation will be on the latest developments in electrospun based scaffolds exhibiting suitable time dependant mechanical properties, nanoscale (fibrous) topography and the ability to deliver locally growth factors. Emerging developments based on tailored combinations of synthetic and natural polymers incorporating nanovectors for local growth factor delivery, related to the EU project P4FiT, will be reported and discussed.

keywords: tendon, bioactive scaffolds, electrospinning

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EPITHELIAL-TO-MESENCHYMAL TRANSITION FOR TENDON REGENERATIVE MEDICINE STRATEGIES

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Epithelial-to-mesenchymal transition (EMT) is a key event in embryo development and post-natal life in which epithelial cells undergo to a transdifferentiation into mesenchymal cells by acquiring a mobile state. This cell transitioning process recognizes the activation of signaling pathways which occur under controlled environments in response to factors controlling stem cell epigenetic reprogramming, self-renewal and differentiation (1). The investigation of EMT processes controlling tissue patterning and organization as well as disregulating healing processes leading to fibrosis, as for tendinopathies, has been addressed for enabling the advancement of regenerative medicine strategies based on the control of EMT-mediated events in generating a favorable local environmental of stem cell-host tissue dialogue as well as of repairing feedback loop between extracellular matrix (ECM) and progenitor/host cells (4).
In order to verify the role exerted by EMT mediated decision during tenogenesis an epithelial stem cell source derived from the amnion were used to analyse the mechanisms underlying the recovery of tendon microarchitecture and function in relation to cell phenotype. To this aim validated in vitro protocols have been used in order to control the phenotype status of AECs before transplantation by obtaining three subset of cells, starting from a unique genome makeup: epithelial (eAECs), mesenchymal (mAECs) (2) and tendon-like (tdAECs) cells (5). The results of this research demonstrate that eAECs and tdAECs are the most suitable phenotypes to use in order to accelerate the process of regeneration in experimental injured tendons. The healing advantage obtained using these two AECs' phenotypes is however obtained through two different underlying mechanisms. Epithelial AEC were able to positively influence the process of tendon healing mainly through the modulation of the host tissue immune environment, targeting a potential shift from pro-inflammatory and pro-fibrotic to pro-regenerative cellular responses, which probably led to a reduced infiltration of inflammatory cells responsible of the ordered deposition of ECM components. On the other hand tdAECs transplantation played the major role in accelerating the deposition and organization of the ECM without strongly influencing the host immune system. Thus, the ability to control the cell phenotype by reproducing the epithelial-mesenchyme-tenodifferentiation stepwise process, allowed us to comprehensively determine how to drive the regenerative process by supporting a favorable stem cell-host tissue cross-talk. Studies that probe the mechanisms underlying the relationship between EMT and EMT/ECM interactions thus represent the next steps forward in elucidating strategies for reducing fibrosis.

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comment: Barboni B. and Russo V. are equally contributing authors.

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keywords: Epithelial-to-mesenchymal transition; Amniotic epithelial stem cells, tendon, regenerative medicine

NEW TOOLS IN TENDON TISSUE ENGINEERING

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The poor healing ability of tendons as well as the limitations of currently used therapies have motivated tissue engineering (TE) strategies to develop living tendon substitutes. At the same time, the significant lack of knowledge on tendon homeostasis and disease mechanism trigger our interest to focus on the development of adequate 3D tissue models that can provide important insights for developing better and innovative therapies. Our team has been exploring the development of cell-laden 3D magnetically responsive systems that recapitulate key features of the native tissue and that can be remotely actuated both during in vitro culture and/or upon in vivo implantation, through the application of external magnetic stimuli. We are exploring conventional and innovative tools such as multimaterial 3D bioprinting to design magnetic responsive systems mimicking specific aspects of tendon tissue architecture, composition and biomechanical properties, which, combined with adequate stem cells, shall render appropriate behavioural instructions to stimulate the regeneration of tendon tissue. We have demonstrated that the magnetic stimulus of different intensities/frequencies can trigger tenonegic differentiation od hASCs and/or modulate inflammatory response of various cell types. Simultaneously, the 3D cell-laden magnetic system are also being as sophisticated 3D tissue models to unravel mechanisms behind tendon homeostasis and repair that shall support the base knowledge to establish rational design criteria for the biofabrication of living tendon substitutes offering the prospect of tendon regeneration as opposed to simple tissue repair.

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MIRNAS AS POTENTIAL REGULATORS OF ENTHESIS HEALING IN A RODENT INJURY MODEL

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Introduction:
MicroRNAs are short, non-coding RNA sequences with the ability to inhibit the expression of a target mRNA at the transcriptional level. MiRNAs are involved in the regulation and modulation of both regenerative and degenerative processes, playing crucial regulatory roles in tissue healing and regeneration. This particular feature makes this family of molecules a very interesting niche to explore in pursuit of novel therapeutic tools in the fields of tissue engineering and regenerative medicine. Among the many tissues comprised in the musculoskeletal system, the tendon-to-bone enthesis is notoriously difficult to treat due to the heterogeneity of its composition. Upon injury, the fibrocartilaginous transition between the tendon and the bony ends of the enthesis usually doesn’t regenerate. Furthermore, the occurring fibrotic process typically yields a scar tissue with poor mechanical properties prone to recurrent rupture. With this study, we aimed to investigate the early expression patterns of fibrosis-related miRNAs in an injured enthesis to select the ultimate miRNA candidate(s) to be used as a therapeutic tool to aid in the treatment of entheses defects.

Methodology:
A longitudinal defect was created at the patellar enthesis of adult rats. Explants were collected at one (n=6) and 10 days (n=6) after the injury. Tissue samples of the contralateral (healthy) side were used as control. MiRNA expression was assessed by a miScript qPCR array specific for fibrosis (Qiagen) containing a total of 86 miRNAs. Fourteen potentially enthesis-injury/regeneration-related miRNAs resulted de-regulated after the injury compared to the expression in the native tissue. The expression of these miRNAs was then validated in each separate sample. Target prediction was carried out by Ingenuity Pathway Analysis (IPA-Qiagen). The expression of the predicted mRNA targets was investigated by qPCR in each sample. Additionally, protein expression levels of the collagens type I, II, III, and X were investigated by western blot analysis.

Results:
We observed that enthesis-injury/regeneration-related miRNAs showed a three- to five-fold down-regulation after one day of the injury and two- to 14-fold up-regulation after 10 days. Further IPA analysis predicted potential mRNA targets relevant for enthesis development and healing for seven of the fourteen de-regulated miRNAs. These were miR-16, -17, -100, -124, -133a, -155 and -182. Furthermore, the predicted mRNA targets included Col2a1, Runx2, Egfr1, Smad2, and Smad3. The mRNA expression pattern confirmed their regulation according to the up- or down-regulation of their respective targeting miRNA. Furthermore, at the protein level, the collagens type I and II resulted down-regulated directly after the injury and up-regulated after 10 days while collagens III and X showed the opposite pattern of expression.

Conclusions:
These preliminary results bring new insights on the role of miRNAs in the early healing phases of the enthesis upon injury. Moreover, miRNA expression can be modulated by using mimics or antagomirs. Therefore, the mimics or antagomir of these de-regulated miRNAs could be used as time-sensitive, therapeutic tools to enhance or inhibit the miRNA modulatory effect over their mRNA target to aid in the regeneration process of the injured enthesis.

keywords: microRNAs, enthesis, tendon, mRNA targets, Collagens

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MULTIMATERIAL AND MULTISCALE SCAFFOLD FOR TENDON/LIGAMENT REGENERATION

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Tendon/ligament injuries are a relevant clinical problem in modern society. Although these tissues can selfheal when a lesion occurs, the complete functional recovery is difficult to achieve due to their low cellularity and vascularity. Moreover, reconstruction strategies have a non-negligible failure rate [1]. Failures often occur at the enthesis, the tendon/ligament-bone insertion [2]. This junction is a heterotypic tissue characterized by a graded structure from soft (ligament) to hard (bone) tissues with a heterogeneous distribution of cell types, matrix components and architecture [3]. In this work, a multimaterial and multiscale approach was developed, to fabricate scaffolds mimicking the complexity of these tissues, exploiting the combination of electrospinning and 3D printing technologies. Firstly, commercial, medical grade, and bioresorbable polymers both natural (porcine gelatin and gelatin methacryloyl(GelMA)) and synthetic (poly(l-lactic acid)(PLLA), poly(lactic-co-glycolic acid)(PLGA), polycaprolactone (PCL)) were systematically investigated to select the most valuable candidate. On supports made by solvent casting, we analyzed cell viability, proliferation, and gene expression of bone marrowderived mesenchymal stem cells (BM-MSCs). Among the tested materials, PLGA and PCL displayed the best ability to promote the proliferation of BM-MSCs. Further studies highlighted the ability of PLGA and PCL to promote, respectively, the tenogenic and osteogenic differentiation of BM-MSCs. Subsequently, a scaffold fabrication protocol was developed.
For the region that interacts with bone, PCL grid-shaped scaffolds were 3D printed by fused deposition modelling (FDM) technology [4]. The ability of these constructs to promote the BM-MSCs osteogenic differentiation was validated by confocal microscopy imaging. The tendon-like aligned fiber network was replicated by electrospinning PLGA fibers collected on a rotating drum collector. Electrospun structures were mechanically tested, and the fiber alignment was evaluated as function of drum revolutions per minute. Viability and proliferation tests highlighted the possibility to use these electrospun structures as scaffold for tendon/ligament engineering. The enthesis was replicated by directly extruding PCL onto PLGA electrospun films. A fine tuning of the extrusion parameters allowed the PCL deposition onto the electrospun PLGA mates without affecting the fibers integrity, as highlighted by scanning electron microscopy analysis. Scaffolds presenting the three different regions were, then, fabricated and the strength of the interface between 3D printed and electrospun structures was evaluated performing tensile tests. Finally, following this fabrication protocol bidimensional and 3D-dimensional anterior cruciate ligament (ACL) scaffolds, presenting the osteotendinous junction, were fabricated.

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keywords: Tissue engineering, Electrospinning, 3D printing, Tendon, Ligament

41883611855

DEVELOPMENT OF LIPID-POLYMER HYBRID NANOPARTICLES FOR TENDON REGENERATION

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Introduction:
Tendinopathies are one of the most common musculoskeletal conditions. Unsatisfactory healing has a significant impact on the life of patients and imposes a remarkable socioeconomic burden. The recovery from tendon injuries is slow and requires extensive rehabilitation. The resulting scar tissue lacks the mechanical integrity of the original tissue, and therefore complete recovery is rarely achieved[1 2]. Several biological therapeutics have been proposed so far, such as the delivery of growth factors, stem cells and recently the application of gene therapy[3], but limited success has been achieved. In this project, lipid-polymer hybrid nanoparticles (LPNs) are proposed for the co-loading of two biological drugs with the aim to achieve tendon regeneration. Interleukin-4 (Il-4) is an anti-inflammatory cytokine extensively used in macrophage polarization towards the anti-inflammatory M2 phenotype. Co-loading of IL-4 with an siRNA against one of the genes involved in fibrosis could potentially render a dual therapeutic effect: immunomodulation and fibrosis prevention [4,5].

Methodology:
LPNs, consisting of a PLGA core and a lipid shell, were prepared using a newly developed method based on nanoprecipitation using a glass-capillary microfluidics technique. Empty LPNs were also analyzed by transmission electron microscopy (TEM) doing negative staining with phosphotungstic acid (PTA). The toxicity of the empty particles was assessed in RAW 264.7 cells with a luminescent CellTiter Glo® assay. The loading of a model siRNA (i.e., eGFP siRNA) in the lipid shell was quantified using the fluorescent Ribogreen assay. The loading of IL-4 in the PLGA core was determined using an ELISA assay. The transfection efficiency of the siRNA-loaded LPNs, measured as eGFP expression inhibition, was evaluated in RAW 264.7 cells expressing eGFP by flow cytometry. The ability of the IL-4-loaded LPNs to polarize macrophages was assessed in the same cell line by quantification of an increased expression of markers of M2 macrophages as compared to M1.

Results:
Homogenous PLGA cores with sizes smaller than 300 nm were obtained by a glass-capillary microfluidics technique upon optimization of the process and formulation parameters. LPNs were prepared by two different microfluidics methods involving one and two steps, respectively, rendering homogenous particles of approximately 350 nm. The TEM images confirmed the results obtained by DLS and unraveled a spherical shape for the PLGA cores and a spherical/elongated shape for the hybrid NPs. LPNs were loaded with eGFP siRNA in the lipid shell and IL-4 in the PLGA core and a lack of toxicity was proved up to concentrations of 200 µg/mL.

Conclusion:
This work demonstrates the potential of hybrid nanoparticles to load biological drugs with

different physicochemical properties and therapeutic effects, allowing for the development of novel nanosystems with dual effects. The developed platform could potentially be used in tendinopathies to promote scarless tissue repair and recovery of the biomechanical function of the tissue.

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keywords: nanoparticles, siRNA, IL-4

S53
Prospects and Challenges in Biological Therapies for Tendon Regeneration
Room: S4 B
(30 Jun 2022, 13:30 - 15:00)

Conveners: Mohammad El Khatib,
Manuela E. Gomes, Denitsa Denitsa

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WHAT INFLUENCES TENDON BIOLOGY?

Britt Wildemann (Experimental Trauma Surgery; Department of Trauma, Hand and Reconstructive Surgery; Jena University Hospital, Jena, Germany)

Impaired tendon function leads to pain and restricted movement of the joints. This impairment can be due to the degeneration of the tendon and, in the worst-case, due to rupture. Conservative and surgical treatments are available, however treatment duration can be long and the failure rate is high. In order to develop new treatment strategies, a better understanding of the processes underlying tendon alterations as well as the factors influencing them is necessary. This talk will give insights into the process of tendon degeneration and biology obtained from human tissue samples and animal studies.

keywords: Tendon; degeneration; biology, influencing factors

94355101524

INFLAMMATION – A CORE FEATURE OF TENDINOPATHIES

Andreas Traweger (Paracelsus Medical University, Salzburg, Austria)

Tendon disorders and injuries are one of the most common musculoskeletal disorders. Our knowledge of the causes and underlying mechanisms for the development of tendinopathies still remain fragmentary. Recent evidence has clearly implicated the presence of immune cells during early tendinopathy and we are beginning to better understand the origin and properties of these cells. Recently, we described tissue-resident cells fulfilling macrophage- or monocyte-related functions in healthy tendons, most likely serving as sentinels which are activated upon tendon tissue injury or pathological stress.
Various intrinsic and extrinsic risk factors have been identified and next to mechanical overuse, other known predisposing factors include rheumatoid arthritis, diabetes, obesity or smoking, all of which elicit or are accompanied by a mild systemic inflammation. We could show that not only local inflammation can affect tendon quality, but the mere presence of a low-grade, allergy-induced systemic inflammation is sufficient to induce structural alterations in tendons and impair tissue function.
In this presentation I will give an overview on the recent advancements in defining the key role of immune-mediated mechanisms in tendon disease.

keywords: tendon, tendinopathy, tendon regeneration, inflammation

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INVESTIGATING INFLAMMATION IN TENDINOPATHY: HOW CAN STEM CELLS HELP US?

Emily Smith (Royal Veterinary College, Hatfield, United Kingdom), Alyce McClellan (Animal Health Trust, Newmarket, United Kingdom), Debbie Guest (Royal Veterinary College, Hatfield, United Kingdom), Jayesh Dudhia (Royal Veterinary College, Hatfield, United Kingdom)

Introduction
Tendon injuries occur commonly in human and equine athletes. Post-injury, the healing response is inadequate leading to increased deposition of scar-tissue and high re-injury rates. This has motivated the development of novel treatments which promote superior tissue regeneration. Particular interest has surrounded the use of bone-marrow mesenchymal stromal cells (BM-MSCs), with clinical investigations showing promising results1. Nevertheless, how BM-MSCs encourage tendon healing is unclear. Tendon injuries invoke an inflammatory response, and although moderate levels of inflammation are required to initiate tendon repair, evidence suggests inadequate resolution of inflammation contributes to fibrotic healing2. Previously, IL-1β was evidenced to exhibit negative effects on equine tenocytes, yet these consequences could be rescued by exogenous IL-1 receptor antagonist protein (IL1Ra)3. In contrast, embryonic stem cell (ESC) derived tenocytes appeared to be protected from the adverse effects of IL-1β, making them the ideal model to investigate tendinopathy further.

Methodology
Three biological replicates of equine adult tenocytes and ESC-tenocytes were cultured with IFN-γ (100 ng/ml), TNFα (10 ng/ml) and IL-1β (1 nM) and/or IL1Ra (100 ng/ml) prior to gene expression analysis and immunocytochemistry to determine inflammatory pathway activation. A 3-D culture model was used to determine the effects of IFN-γ, TNFα and IL-1β on collagen gel contraction by adult or ESC-tenocytes over 14 days. Adult tenocytes were also stimulated with IFN-γ, TNFα and IL-1β alone or in co-culture with BM-MSCs or BM-MSC conditioned media and the effects on signal induction, gene expression and 3-D collagen gel contraction measured.

Results
Stimulation of adult tenocytes with IFN-γ, TNFα and IL-1β resulted in significant changes in tendon-associated gene expression. Furthermore, these cytokines significantly inhibited 3-D collagen gel contraction by adult tenocytes. Immunocytochemistry demonstrated this combination of cytokines activated NF-κB, but not STAT1, JNK or P38 MAPK in adult tenocytes. These adverse effects could not be rescued by IL1Ra or factors produced by BM-MSCs. Conversely, ESC-tenocytes appear to be protected from IL-1β, TNFα and IFN-γ; generating tendon-like constructs indistinguishable from controls and demonstrating little to no changes to gene expression. Additionally, inflammatory stimulation failed to activate key inflammatory signalling pathways in ESC-tenocytes.

Conclusion
We demonstrate IL-1β, TNFα and IFN-γ work synergistically to induce greater detrimental consequences for adult tendon function than when used individually. Moreover, these adverse effects cannot be rescued by direct suppression of IL-1β. However, ESC-tenocytes appear to be protected from inflammatory stimulation, exhibiting minimal effects on gene expression and no activation of NF-κB, suggesting an association between undesired cellular activities in tenocytes and NF-κB signalling. Our results suggest BM-MSCs are unable to protect adult tenocytes from the adverse effects of inflammation. Understanding the mechanisms by which NF-κB signalling

is blocked in ESC-tenocytes, and how BM-MSCs facilitate healing, may enable us to identify novel interventions for tendon injuries which minimise scar-tissue formation, resulting in diminished re-injury rates and improved quality of life.

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keywords: Tendon, Regeneration, Inflammation

41883627366

MAGNETIC NANOPARTICLE-MEDIATED ORIENTATION OF COLLAGEN HYDROGELS FOR IN VITRO MODELLING AND REGENERATIVE THERAPIES

Abigail Wright (University of Birmingham, Birmingham, United Kingdom), Lucrezia Righelli (University of Birmingham, Birmingham, United Kingdom), Thomas Broomhall (University of Birmingham, Birmingham, United Kingdom), Hannah Lamont (University of Birmingham, Birmingham, Poland), Alicia El Haj (University of Birmingham, Birmingham, United Kingdom)

Despite the high incidence of tendon injuries globally, an optimal treatment strategy has yet to be defined1. A key challenge for tendon repair is the alignment of the repaired matrix into orientations which provide maximal mechanical strength2, 3. Using oriented implants for tissue growth combined with either exogenous or endogenous stem cells may provide a solution. Previous research has shown how oriented fiber-like structures within 3D scaffolds can provide a framework for organized extracellular matrix deposition4. In this paper, we present our data on the remote magnetic alignment of collagen hydrogels which facilitates long-term collagen orientation. Magnetic nanoparticles (MNPs) at varying concentrations and size can be contained within collagen hydrogels. In the presence of an external magnetic field, gelation is initiated by incubation at 37oC for 30-minutes. Our data shows how, in response to the magnetic field lines, MNPs align and form string-like structures orientating 90o from the applied magnetic field from our device. This can be visualized through light and fluorescence microscopy and persists 21-days post application of magnetic field. Confocal microscopy demonstrates anisotropic macroscale structure of MNP-laden collagen gels subjected to a magnetic field, compared to gels without MNP dosing. Matrix fibrillation was compared between non- and biofunctionalized MNP hydrogels, and different gels dosed with varying MNP concentrations. Human adipose stem cells (hASCs) seeded within the magnetically-aligned gels were seen to align in parallel to MNP and collagen orientation 7-days post application of magnetic field. hASCs seeded in isotropic gels were randomly organized. To summarize, we have developed a convenient, non-invasive protocol to control collagen I hydrogel architecture. Through the presence or absence of MNP dosing and a magnetic field, collagen can be remotely aligned or randomly organized, respectively, in situ. This can be considered as an innovative approach particularly useful in tissue engineering or organ-on-a-chip applications for remotely controlling collagen matrix organization. In this way cellular constructs analogous to healthy and diseased tendon can be engineered ex vivo for regenerative therapies.

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keywords: magnetic nanoparticles, tendon, tissue engineering, collagen

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PRO-RESOLVING MEDIATORS IN ROTATOR CUFF TENDINOPATHY: HOW IS THE BURSA INVOLVED?

Franka Klatte-Schulz (Julius Wolff Institute and BIH Center for Regenerative Therapies, BIH at Charité-Universitätsmedizin Berlin, Berlin, Germany), Nicole Bormann (Julius Wolff Institute and BIH Center for Regenerative Therapies, BIH at Charité-Universitätsmedizin Berlin, Berlin, Germany), Aysha Schmock (Julius Wolff Institute and BIH Center for Regenerative Therapies, BIH at Charité-Universitätsmedizin Berlin, Berlin, Germany), Jasmin Al-Michref (Julius Wolff Institute, BIH at Charité-Universitätsmedizin Berlin, Berlin, Germany), Kathi Thiele (Center for Musculoskeletal Surgery, Charité-Universitaetsmedizin Berlin, Berlin, Germany), Philipp Moroder (Center for Musculoskeletal Surgery, Charité-Universitaetsmedizin Berlin, Berlin, Germany), Birgit Sawitzki (Institute of Medical Immunology and Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Germany, Berlin, Germany), Britt Wildemann (Experimental Trauma Surgery, Department of Trauma-, Hand- and Reconstructive Surgery, University Hospital Jena, Germany, Jena, Germany), Georg N. Duda (Julius Wolff Institute and BIH-Center for Regenerative Therapies, Berlin Institute of Health at Charité-Universitaetsmedizin Berlin, Berlin, Germany)

A well-balanced shift from pro- to anti-inflammatory processes during healing appears essential for successful regeneration and may prevent from chronic diseases or tissue damage. Resolution of inflammation is an active process, which is regulated by specialized pro-resolving mediators (SPMs) such as lipoxins, annexins, and resolvins. The contribution of these mediators to the severity of rotator cuff (RC) tendinopathy has previously been shown. Due to the close localization of RC tendons to the subacromial bursa, we hypothesize that bursae may store pro-resolving mediators to regulate tendon regeneration. We aimed to detect differences at increasing stages of RC tendinopathy in order to identify mediators that may regulate its deterioration.

We investigated bursae from healthy patients (n=13-16) versus patients with partial (n=13-17) or full-thickness RC tears (n=18-32). Bursa tissues were harvested for immunofluorescence staining, gene expression analysis and release of SPMs (Annexin A1, Lipoxin A4, Resolvin D1/D2) and/or their receptors (FPR2, ChemR23, GPR18). To understand regulatory mechanisms of SPM signaling in bursae, bursa-derived cells from patients with full-thickness RC tears (n=6) were subjected to physiological (2%) or pathological (8%) straining under uniaxial cyclic loading for 4 hours/day on 3 consecutive days. After stimulation, cells were analyzed by flow cytometry for SPM receptors (FPR2, ChemR23), fibroblast markers (CD90, CD105), adhesion molecules (CD54, CD106), human leucocyte antigens (HLA-DR, HLA-ABC) and proliferation marker Ki67. Furthermore, gene expression of SPM related genes (Annexin A1, FPR1, FPR2, ChemR23, GFP18), Col I, matrix degrading enzymes and inhibitors (MMP1, -2, TIMP1), and pro-inflammatory cytokines (IL6, IL1b) were analyzed.

Multiplex fluorescence staining revealed FPR2+ and ChemR23+ cells in perivascular, but also in fibrous or fatty bursa tissue. Single or double positive FPR2/ChemR23 cells were identified partially as CD45+ leukocytes but also as CD45- cells (e.g. endothelial cells, fibroblasts). Annexin A1 gene expression showed a trend towards an increase in bursae of partial RC tears compared to full-thickness RC tears (p=0.076), whereas SPM receptor gene expression was not significantly affected. In tissue culture, bursae of partial RC tears showed increased Annexin A1 and Resolvin D1 release compared to bursae of intact controls (p=0.029, p=0.007, respectively). Resolvin D1 release was also increased in bursae of full-thickness RC tears compared to intact controls (p=0.008). Pathological, but not physiological mechanical straining resulted in significantly

increased MMP1 and ChemR23 gene expression compared to unstimulated controls (p=0.002, p=0.040). On surface marker level, no significant regulations were observed after mechanical stimulation of bursa-derived cells.
The study shows for the first time SPM signaling mediators in subacromial bursa tissue with differences depending on the severity of RC tears. The increase in Annexin A1 and Resolvin D1 particularly in early tendinopathy (partial RC tear) may indicate that these bursae are trying to balance the pro-inflammatory response at this stage. Pathological mechanical straining induced ECM remodeling, due to strongly increased MMP1 gene expression and might be an initiator of a pro-resolving response, as ChemR23 gene expression was up-regulated. In summary, the results provide first evidence that the subacromial bursa is involved in pro-resolving processes in RC tendinopathy.

keywords: subacromial bursa, tendinopathy, pro-resolving mediators, mechanical stimulation

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HUMAN 3D TENDON-ON-CHIP MODEL TO INTERROGATE THE MULTICELLULAR CROSSTALK IN TENDINOPATHY

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INTRODUCTION
Tendon tissues host different cell populations that play important roles in their physiology and pathophysiology. A hallmark of tendon injuries and diseases is the persistent inflammatory response that can self-amplify and lead to chronicity. The inflammatory phase of tendinopathy is characterized by increased vascularization and influx of immune cells (mast cells, macrophages, T cells) at the healing site. A better understanding of this complex multicellular crosstalk and environmental cues is critical for decoding the healing mechanisms of tendon injuries and to find new therapeutic options. However, the current lack of representative in vitro models of tendinopathy is a major barrier to the progress of this field. The aim of this work is to establish a multicellular organotypic 3D model recreating key signaling hallmarks of that immune response, in particular for the study of the interactions between stromal tenocytes and the circulating T cells in tendon vasculature under healthy and diseased conditions.

METHODOLOGY
To recreate the anisotropic fibrillar architecture of tendon ECM and induce cell alignment in 3D within the chip, we produced magnetically responsive microfibers (MNF@PCL). Microfibes were prepared by cryo-sectioning electrospun PCL meshes incorporated with iron oxide nanoparticle. A three channeled microfluidic chip was used as platform to build the model, where human tendon derived cells (hTDCs) were encapsulated in the central channel in either transglutaminase crosslinked Gelatin or Platelet Lysate (PL) hydrogels along with MNF@PCL. For in-situ alignment of MNF@PCL, the chip was placed under a uniform magnetic field created by two parallel magnets. Hydrogel formation allowed to fix the fiber pattern after removal of the magnetic forces. Microvascular cells were co-cultured in the side channel to recreate the open vasculature of the extrinsic tendon compartment, where T cells can be subsequently circulated for evaluating their interactions with stromal tenocytes.

RESULTS
Analysis of 3D hTDCs cytoskeleton organization within hydrogel matrices showed that the topographical cues created by the microfiber alignment strongly dictates the cell's aspect ratio and orientation. The synergy between the PL matrix bioactivity and magnetically aligned MNF@PCL revealed to be the most effective strategy for inducing cell anisotropic organization within central compartment and maintenance of a tenogenic phenotype. The microvascular cells co-cultured in the side channels organized into compartmentalized tubular monolayer with open lumen. We are currently assessing the effects stemming from the crosstalk between tendon and vascular cells on genes and proteins related with ECM, tenogenic markers and inflammatory signaling pathways. This physiomimetic system is also being explored to study the effects of hTDCs on the behavior of circulating T cells (migration and activation), as well as the impact of these crosstalk mechanisms on the stromal compartment.

CONCLUSION
In this work, we propose a compartmentalized tendon-on-chip model able to recapitulate ex-vivo some of the characteristic microstructural features of healthy and diseased (fibrotic) tendon stroma interfacing with the vasculature of the extrinsic tendon compartment which is capable of supporting circulating immune cells. This physiomimetic system is being leveraged for better understanding not only the mechanisms of tendinopathy, but also of tendon tissue regeneration and repair.

comment:ERC Grant CoG MagTendon nr 772817; FCT for the Ph.D. grant of SMB (PD/BD/129403/2017), contract to MGF (CEECIND/01375/2017) and RD (2020.03410.CEECIND); and project SmarTendon (PTDC/NAN-MAT/30595/2017). AP for his postdoctoral grant ED481B2019/025.

keywords: tendon, organ-on-chip, ex-vivo modeling, immune cells

S54+S14
Regulation of cell phenotype in osteochondral tissues: towards RNA therapy for bone and cartilage repair + Biological testing of 3D-printed biomaterials – towards updated norms
Room: S2
(1 Jul 2022, 11:00 - 12:30)

Conveners: Veronika Hruschka, Marley Dewey, Andrea Lolli, Eric Farrell, Carlos Julio Peniche Silva, Daniel Seitz

62903402364

CARTILAGE AND BONE REGULATION BY MICRORNAS

David Young (Newcastle University, Newcastle Upon Tyne, United Kingdom)

MicroRNAs, short non-coding RNAs, are important regulators of skeletal development. Several individual microRNAs have been shown to alter long bone growth with most interest focused on the cartilage ‘specific’ microRNA, miR-140. We made a new miR-140-null mouse and confirmed the skeletal phenotype, identifying a range of target genes that the microRNA controls. The null mice also have increased joint damage during surgically-induced osteoarthritis and cartilage transcriptome analysis has revealed novel pathways in which the microRNA functions during joint destruction.

Less is known about microRNAs and bone development, though serum microRNAs are biomarkers for osteoporosis. We identified a little studies microRNA that appears important in bone ageing. Mice null for this microRNA develop bone thickening with age, with increased cortical and trabecular bone thickening. In the talk I will discuss how this one microRNA fine tunes osteoblast, osteoclast and osteocyte transcriptomes and thus function to regulate bone formation and turnover.

keywords: bone, microRNA, osteoblast, cartilage

31451703755

MRNA THERAPEUTICS FOR MUSCULOSKELETAL TISSUE HEALING

Emily Smith (Royal Veterinary College, Hatfield, United Kingdom), Alyce McClellan (Animal Health Trust, Newmarket, United Kingdom), Debbie Guest (Royal Veterinary College, Hatfield, United Kingdom), Jayesh Dudhia (Royal Veterinary College, Hatfield, United Kingdom)

Introduction
Tendon injuries occur commonly in human and equine athletes. Post-injury, the healing response is inadequate leading to increased deposition of scar-tissue and high re-injury rates. This has motivated the development of novel treatments which promote superior tissue regeneration. Particular interest has surrounded the use of bone-marrow mesenchymal stromal cells (BM-MSCs), with clinical investigations showing promising results1. Nevertheless, how BM-MSCs encourage tendon healing is unclear. Tendon injuries invoke an inflammatory response, and although moderate levels of inflammation are required to initiate tendon repair, evidence suggests inadequate resolution of inflammation contributes to fibrotic healing2. Previously, IL-1β was evidenced to exhibit negative effects on equine tenocytes, yet these consequences could be rescued by exogenous IL-1 receptor antagonist protein (IL1Ra)3. In contrast, embryonic stem cell (ESC) derived tenocytes appeared to be protected from the adverse effects of IL-1β, making them the ideal model to investigate tendinopathy further.

Methodology
Three biological replicates of equine adult tenocytes and ESC-tenocytes were cultured with IFN-γ (100 ng/ml), TNFα (10 ng/ml) and IL-1β (1 nM) and/or IL1Ra (100 ng/ml) prior to gene expression analysis and immunocytochemistry to determine inflammatory pathway activation. A 3-D culture model was used to determine the effects of IFN-γ, TNFα and IL-1β on collagen gel contraction by adult or ESC-tenocytes over 14 days. Adult tenocytes were also stimulated with IFN-γ, TNFα and IL-1β alone or in co-culture with BM-MSCs or BM-MSC conditioned media and the effects on signal induction, gene expression and 3-D collagen gel contraction measured.

Results
Stimulation of adult tenocytes with IFN-γ, TNFα and IL-1β resulted in significant changes in tendon-associated gene expression. Furthermore, these cytokines significantly inhibited 3-D collagen gel contraction by adult tenocytes. Immunocytochemistry demonstrated this combination of cytokines activated NF-κB, but not STAT1, JNK or P38 MAPK in adult tenocytes. These adverse effects could not be rescued by IL1Ra or factors produced by BM-MSCs. Conversely, ESC-tenocytes appear to be protected from IL-1β, TNFα and IFN-γ; generating tendon-like constructs indistinguishable from controls and demonstrating little to no changes to gene expression. Additionally, inflammatory stimulation failed to activate key inflammatory signalling pathways in ESC-tenocytes.

Conclusion
We demonstrate IL-1β, TNFα and IFN-γ work synergistically to induce greater detrimental consequences for adult tendon function than when used individually. Moreover, these adverse effects cannot be rescued by direct suppression of IL-1β. However, ESC-tenocytes appear to be protected from inflammatory stimulation, exhibiting minimal effects on gene expression and no activation of NF-κB, suggesting an association between undesired cellular activities in tenocytes and NF-κB signalling. Our results suggest BM-MSCs are unable to protect adult tenocytes from the adverse effects of inflammation. Understanding the mechanisms by which NF-κB signalling

is blocked in ESC-tenocytes, and how BM-MSCs facilitate healing, may enable us to identify novel interventions for tendon injuries which minimise scar-tissue formation, resulting in diminished re-injury rates and improved quality of life.

References
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keywords: Tendon, Regeneration, Inflammation

52354524255

CHROMATIN COMPACTION DECREASES CELL ADHESION STRENGTH: AN ANALYSIS BY FLUIDIC FORCE MICROSCOPY

Julie Buisson (University of Strasbourg / Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg, Strasbourg, France), Xinyu Zhang (Laboratory of Biosensors and Bioelectronics, Department of Information Technology and Electrical Engineering, ETH Zürich, Zürich, Switzerland), Pierre Schaaf (Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg (CRBS), Strasbourg, France), Philippe Lavalle (Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Strasbourg, France), Tomaso Zambelli (Laboratory of Biosensors and Bioelectronics, Department of Information Technology and Electrical Engineering, ETH Zürich, Zürich, Switzerland), Dominique Vautier (Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Strasbourg, France), Morgane Rabineau (Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Strasbourg, France)

Background
Cell adhesion can be guided through the mechanical signals provided by the biomaterials1,2. These signals are transmitted to the cytoskeleton, to the nuclei and finally to the chromatin3. The chromatin remodeling is highly dynamic and sensitive to these cues and imposes extensive effects on DNA-related metabolism, including transcription. Recently, chromatin has been identified as a mechanosensitive compartment that is shaped as much by external forces pressing down upon it, as internal forces pushing outwards from the chromatin: this dynamic process activate or silence genes. Chromatin therefore has a major role in the fate of the cell, in particular for the stem cell. Inversely, how chromatin communicates cues back to the nuclear membrane is poorly understand. Particularly, whether chromatin structure impacts by itself cell adhesion, more precisely the cell adhesion strength, is an underexplored topic.

Methods
We have investigated if hyper-condensation of chromatin by ATP depletion (with sodium azide and 2-deoxyglucose, SA2D) or by inhibition of histone acetyltransferases (with anacardic acid, ANA) alters epithelial cell adhesion strength. This adhesion force is measured by fluidic force microscope (FluidFM), an atomic force microscope-driven micropipette4. Through this induced remodeling of chromatin, we investigated the organization of the cytoskeleton and focal adhesion.

Results
Chromatin compaction within nuclei is induced by SA2D or by ANA. Our results demonstrate that this phenomenon is accompanied by a decrease of chromatin acetylation, an increase of histone H3K27 methylation and a decrease of the nuclear area. This leads to reorganization of the actin cytoskeleton showing small stress fibers localized around the nucleus while focal adhesion contacts decrease in size compare to untreated cells. By videomicroscopy, we show that after drugs remove from to the culture medium, cells morphologic are restored with nucleus decondensed. We observed by FluidFM that chromatin compaction by SA2D or ANA significantly decreases cell adhesion strength compared to untreated cells.

Conclusions
These results show for the first time that structure of the chromatin physically impact cell adhesion strength. Moreover, this chromatin remodeling is correlated with a global

deacetylation of the nucleus and impact the dynamic of the cytoskeleton and the focal adhesion contacts.
A new open question is to determine how cell adhesion strength is mechanically impacted by chromatin structure, especially during stem cell differentiation and thus could provide essential data in epigenetic cell reprogramming for stem cell tissue engineering. Another attractive question will be to determine how a cell rapidly coordinates external forces with internal genomic forces to accomplish mechanosensitive biological processes.

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keywords: Chromatin mechanobiology, Chromatin hyper-condensation, Epigenetic modifications, Cell adhesion strength, FluidFM

52354515324

IMPROVING CHONDROGENIC POTENTIAL OF MESENCHYMAL STROMAL CELLS BY SIRNA DELIVERY IN HYDROGELS.

Guoliang Chen (AO Research Institute Davos and Sun Yat-sen University, Shenzhen, China, Davos Platz, Switzerland), Valentina Basoli (AO Research Institute Davos, Davos Platz, Switzerland), Anne Géraldine Guex (AO Research Institute Davos, Davos Platz, Switzerland), Martin Stoddart (AO Research Institute Davos, Davos Platz, Switzerland), Elena Della Bella (AO Research Institute Davos, Davos Platz, Switzerland)

It is well known that cartilage possesses a low intrinsic regenerative potential, causing tissue damage that remains unhealed and contributes to a high socioeconomical burden for affected patients. New strategies to restore the properties of load bearing, friction-reducing hyaline cartilage are thus timely. Tissue engineering and regenerative medicine approaches must deal with the high donor-to-donor variability typical of primary human cells, including bone marrow derived-mesenchymal stromal cells (BMSCs). Recently, we have demonstrated that the chondrogenic potential of BMSCs can be predicted on a donor-specific basis by the ratio between the gene expression levels of two main TGF-β receptors, namely TGFBR1 and TGFBR2 [1]. Also, a transient downregulation of the TGF-β receptors TGFBR2 and ACVRL1 was sufficient to reverse the phenotype of cells that poorly responded to TGF-β1 stimulation and increased their chondrogenic commitment in pellet culture. In the field of regenerative medicine, major efforts focus on engineering hyaline cartilage of clinically relevant sizes which, in addition to the cell type, requires an adequate 3-dimensional cell culture substrate or scaffold. Therefore, the aim of this study is to validate the translational potential of those findings and explore the feasibility of siRNA delivery in hydrogels to improve chondrogenic differentiation of human BMSCs.

For this purpose, human BMSCs were isolated and expanded from the bone marrow of patients undergoing spinal surgery, with full ethical approval (Bern Req-2016-00141) and written informed consent. Gelatin methacryloyl (GelMA) was synthesized using Gelatin type A from porcine skin and methacrylic anhydride to reach a 50% degree of substitution. Gels were prepared as 8% w/v GelMA in PBS containing 0.3% Irgacure and cured at 365 nm. Fibrin was prepared using Fibrinogen and Thrombin from human plasma (Sigma-Aldrich) to a final concentration of 25 mg/ml fibrinogen and 1 U/ml thrombin, respectively. Cells were encapsulated in either GelMA or fibrin at a density of ~20x106 cells/ml. For siRNA delivery, gels were supplemented with either a negative control or a TGFBR2-targeting siRNA (10 pmol, Thermo Fisher) complexed with Fuse-It-siRNA reagent (Ibidi). Constructs were cultured for up to 3 days for RNA isolation and RT-qPCR analysis. Samples were stained with calcein green and ethidium homodimer for analysis of cell viability at 7 days.

Confocal microscopy was used to evaluate the Live/Dead staining of constructs after 7 days in culture, showing good viability and even distribution of embedded cells. Up to 3 days, gene expression analysis from fibrin constructs showed a consistent downregulation of TGFBR2, resulting in an increase of the TGFBR1/TGFBR2 ratio. This phenomenon was much less pronounced in GelMA, potentially because of the lower migration ability of cells within this hydrogel. Future evaluations will explore the chondrogenic differentiation potential of distinct BMSC donors within different hydrogels and in response to siRNA delivery. We suggest that this method will increase the therapeutic efficacy of patient-specific cell-hydrogel based constructs for cartilage regeneration.

The work is supported by AO Foundation and AO Research Institute Davos. GC was funded by the Chinese-Swiss Young Researchers' Exchange Programme (EG-CN_04-032019).

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keywords: cartilage, mesenchymal stromal cells, siRNA, hydrogels, TGFBR2

20941831157

3D PRINTING OF SOL-GEL SILICA-BASED HYBRIDS FOR BONE REGENERATION

Raquel Rodriguez-Gonzalez (Bioengineering Insitute of Technology (BIT)- Universitat Internacional de Catalunya, Barcelona, Spain), Roman Perez Antoñanzas (Bioengineering Insitute of Technology (BIT)- Universitat Internacional de Catalunya, Barcelona, Spain), Luis Delgado Garoña (Bioengineering Insitute of Technology (BIT)- Universitat Internacional de Catalunya, Barcelona, Spain)

Introduction
Silica based materials have been commonly studied in the field of bone regeneration, due to their bioactivity and osteogenic properties1. However, silica materials have low degradation rates and are brittle, which can be overcome by developing hybrid materials, which include an organic part bound to the silica network2. Despite these can be processed in several shapes, there is a need to fabricate these materials in a custom made morphology to adapt better to the site of defect, producing scaffolds with a desired porosity, shape and size3. However, 3D printing of silica materials normally uses very high temperatures to obtain the final structure which limits the ability to encapsulate bioactive molecules. An alternative to produce silica based materials at low temperatures is the sol-gel method, in which a precursor forms a silica network using pH or temperature as a catalyst4. Therefore, in this study, a printable sol-gel silica-based hybrid has been developed by combining tetraethylorthosilicate (TEOS), and gelatin that were cross-linked with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS).

Methodology
The inks were prepared by adjusting the amount of TEOS and GPTMS in the sol and the proportion of gelatin added and mixing them at 37°C to obtain printable inks. The developed inks were 3D printed using a Cellink BioX printer, and the different physicochemical properties were analyzed. As control, pristine printed silica was used. The analyzed properties were shape fidelity, water uptake, degradation, mechanical properties and bioactivity. Cytotoxicity and proliferation were assessed using rat mesenchymal stem cells, counting the cells with a PicoGreen assay and observing the morphology with an immunofluorescence assay. In vitro osteogenic differentiation was also assessed using rMSCs and osteogenic media, using pristine silica and PLA as controls. The differentiation was measured by ALP assay. Statistical significance was accepted at $p<0.05$.

Results
Three different inks were obtained, one of them with a high amount of cross-linker (HC) and two with a lower amount (LC). All inks maintained the shape fidelity of the scaffold design. Regarding the water uptake, degradation, bioactivity and mechanical properties, all of the hybrid conditions showed an improvement when comparing with pristine silica. HC showed cytotoxic effects, but not the LC conditions, which indicates the potential toxicity of the cross-linker. Conditions with gelatin showed an improved adhesion and proliferation of the cells, comparing with the pristine silica. The differentiation showed that the hybrids have lower osteogenic properties than pristine silica, however, when comparing with PLA scaffolds, it showed that, even though the gelatin decreases the ALP activity, it still promotes osteogenic differentiation.

Conclusion
Three different silica-gelatin 3D printable hybrids were obtained. The hybrid inks improved all physicochemical properties when comparing to pristine silica scaffolds. Moreover, the cell

adhesion and proliferation was improved by hybrid scaffolds, maintaining the osteogenic activity of these silica-gelatin hybrid scaffolds.

References

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keywords: Bone, Silica, 3D printing, Hybrid, GPTMS

S55
REMODELing the Future: next generation of organoid models for biomedicine
Room: S4 A
(30 Jun 2022, 11:00 - 12:30)

Conveners: Marta Alves Da Silva,
Silvia Maria Mihăilă

31451703204

BIOENGINEERING VASCULARIZED MICROTISSUES

Cristina Barrias (i3S/INEB-Instituto de Investigação e Inovação em Saúde/Instituto de Engenharia Biomédica, Porto, Portugal)

Modular tissue-engineering approaches provide a promising strategy for building complex living structures from the bottom-up, through the co-assembly of microscale tissue units. Using biofabrication tools, multiple modular units of parenchymal, stromal, and vascular tissues can be rationally combined to recreate structurally/functionally different compartments of human organs. Microtissue units present a high surface area, which facilitates the diffusion/mobility of oxygen, molecules, and cells through interstitial gaps, affording a useful tool to generate densely cellularized 3D constructs. In this talk, we outline different approaches to engineering vascularized microtissues and describe some of their applications in the fields of regenerative medicine and in vitro modeling.

keywords: bottom-up tissue engineering, microtissue, vascularization

31412708526

COMBINING CHOLANGIOCARCINOMA ORGANOIDS AND DECELLULARIZED LIVER SCAFFOLDS UNVEILS MICROENVIRONMENT-DEPENDENT EXTRACELLULAR MATRIX REMODELING

Gilles Van Tienderen (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Oskar Rosmark (Lung Biology, Department of Experimental Medical science, Lund University, Lund, Sweden), Ruby Lieshout (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Jorke Willemse (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Floor De Weijer (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Linda Elowsson Rendin (Lung Biology, Department of Experimental Medical science, Lund University, Lund, Sweden), Gunilla Westergren-Thorsson (Lung Biology, Department of Experimental Medical science, Lund University, Lund, Sweden), Michail Doukas (Department of Pathology, ErasmusMC, University Medical Center Rotterdam, Rotterdam, Netherlands), Bas Groot Koerkamp (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Martin Van Royen (Department of Pathology, ErasmusMC, University Medical Center Rotterdam, Rotterdam, Netherlands), Luc Van der Laan (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands), Monique Verstegen (Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands)

Background and Aims:
Cholangiocarcinoma (CCA) is a highly aggressive tumor which arises from the biliary duct epithelium. Currently available models fail to recapitulate the full complexity of CCA, particularly the desmoplastic environment and the interplay between cancer cells and the extracellular matrix (ECM). We aimed to create an improved 3D in vitro model by combining patient-derived CCA organoids (CCAOs) with native CCA tumor and liver ECM, obtained by decellularization, to study the role of tumor cells in desmoplasia and ECM remodelling.

Method:
Patient-derived CCA matrix (CCA-M) and tumor-free liver matrix (TFL-M) were obtained by decellularization of tumor and tumor-free liver biopsies from CCA patients. The decellularized scaffolds were biochemically and mechanically assessed using nanoindentation and rheology. Subsequently, CCA-M and TFL-M were recellularized with CCAOs. Tumor cell behavior of CCAOs in CCA-M, TFL-M was studied on a transcriptome level with bulk RNA-sequencing, and protein level with immunocytochemical stainings and Stable Isotope Labeling by Amino acids in Cell culture (SILAC)-based mass spectrometry. Cell viability measurements were taken for quantifying CCAO response to chemotherapeutics. Standard culture conditions of CCAOs in basement membrane extract (BME) were used as control.

Results:
Decellularization of CCA tumor and liver tissue resulted in effective removal of cells while preserving ECM structure and retaining important characteristics of the tissue origin, including stiffness, collagen content, and the presence of desmoplasia, typically associated with CCA, in the tumor ECM. When culturing CCAOs in CCA-M, the expression profile of differentially expressed genes much more resembled the transcriptome of primary CCA tumor tissue in vivo compared to TFL-M (correlation coefficient (CC) CCA-M 0.83±0.03 vs CC TFL-M 0.70±0.03, $p = 0.004$) and BME (CC CCA-M 0.88±0.04 vs CC BME 0.63±0.06, $p < 0.0001$). This was accompanied by a significant difference in cell viability in response to exposure to gemcitabine, which is the standard of care treatment for CCA patients (mean viability at 100uM CCA-M 0.86 vs TFL-M 0.64, $p = 0.018$). These results provide evidence that the desmoplastic extracellular environment in CCA plays an important role in chemoresistance. Moreover, CCA-M induced specific extracellular matrix protein production in CCAOs, such as fibronectin 1 (FN1), which is related to desmoplasia and decreased patient survival. In TFL-M, lacking desmoplasia, CCAOs initiated a desmoplastic reaction directly through increased production of multiple collagen types (e.g. COL1A1, COL1A2, COL6A1, COL6A3).

Conclusion:
This study demonstrates that combining tumor organoids and decellularized matrix provides a complex in vitro tumor model that can recapitulate key components of CCA biology, including transcriptome profiles, drug responses, and ECM remodeling activity. The increased production of ECM proteins, primarily collagens, indicates that epithelial tumor cells are able to contribute to their own desmoplastic environment. Complementing organoid-based culture models with tumor decellularized matrix is applicable to a variety of tumors and could result in overall better recapitulation of tumor behavior in vivo.

keywords: Decellularization, Cholangiocarcinoma, Extracellular matrix remodelling, organoids

94238154186

MICROENGINEERED SYSTEM TO GENERATE THE FUNCTIONAL INNER EAR ORGANOIDS WITH ENHANCED UNIFORMITY AND MATURITY

Sunho Park (Chonnam National University, Gwangju, South Korea), Yeon Ju Kim (Ajou University School of Medicine, Suwon, South Korea), Yonghyun Gwon (Chonnam National University, Gwangju, South Korea), Woochan Kim (Chonnam National University, Gwangju, South Korea), Hyosung Kim (Chonnam National University, Gwangju, South Korea), Yun-hoon Choung (Ajou University School of Medicine, Suwon, South Korea), Jangho Kim (Chonnam National University, Gwangju, South Korea)

Inner ear disorders (e.g., hearing loss) are common, but it is difficult to develop a therapeutic drug and to find a specific mechanism because of a lack of the research platforms. Inner ear organoids (IEO) are perceiving as an innovative research platform to reproduce the complex inner ear systems and to solve the previous problems. To improve uniformity and reproducibility of IEO, we develop a microengineered system that can collect cells and form aggregates rapidly. Using microengineered system, we can easily control the size of aggregates by the initial cell number and find the optimal condition to develop mature IEO. Compared with the traditional approach to develop IEO (i.e., IEO grown on U well plate; U-IEO), it is possible to develop IEO with the mass production and more reproducible shape. In addition, IEO grown on microengineered system (M-IEO) have an improved functions including the formation of mature kinocilia and the electrophysiological function than U-IEO. Thus, we conclude M-IEO may have great potential to overcome the limitations of the traditional approach, and it may be used as an advanced platform for inner ear disorder.

keywords: Inner Ear Organoid, Microengineering, Stem Cells

20941849689

SYNTHETIC SUPRAMOLECULAR HYDROGELS FOR THE 3D CULTURE OF KIDNEY EPITHELIAL CELLS AND INTESTINAL ORGANOIDS

Laura Rijns (TU/e, Eindhoven, Netherlands), Joost Wijnakker (Hubrecht Institute Utrecht, Utrecht, Netherlands), Wim De Lau (Hubrecht Institute Utrecht, Utrecht, Netherlands), Marjan Hagelaars (TU/e, Eindhoven, Netherlands), Joris Peeters (SyMO-Chem bv, Eindhoven, Netherlands), Henk Janssen (SyMO-Chem bv, Eindhoven, Netherlands), Sandra Loerakker (TU/e, Eindhoven, Netherlands), Carlijn Bouten (TU/e, Eindhoven, Netherlands), Hans Clevers (Hubrecht Institute Utrecht, Utrecht, Netherlands), Bert Meijer (TU/e, Eindhoven, Netherlands), Patricia Dankers (TU/e, Eindhoven, Netherlands)

Introduction:
An abundant and important receptor that regulates numerous ECM-cell interactions is the integrin receptor. To illustrate, integrins influence the cells' polarity by controlling the apical-basal orientation. Kidney epithelial cells and intestinal organoids cultured in suspension and thus in the absence of matrix, invert the direction of their polarization, with an apical membrane facing outwards. These observations raise the question whether direct control over the integrins via the matrix' stiffness and degree of bioactivity could possibly control the orientation of polarization, thereby directing epithelial morphogenesis and controlling organoid behavior. Therefore, we propose synthetic, modular supramolecular hydrogels based on the ureido-pyrimidinone (UPy) motif, which use directional, non-covalent interactions. These assemblies are eminently suitable to study cell-material interactions, because herein we have full and independent control over several different properties, like stiffness and ligand concentration, allowing that the effects of such microenvironment components can be assessed individually. The UPy hydrogels consist of 3 molecules: monofunctional (M), bifunctional (B) and bioactive (RGD) UPys. Herein, the M UPys can form one-dimensional fibers, while the B UPys could act as a crosslinker between the M UPys to create a network with adjustable mechanical properties, by changing the M/B UPy ratio or by varying the hydrogel's concentration. And finally, RGD UPys could be mixed in as integrin-binding ligands.
Here, we aim to investigate the influence of stiffness on renal epithelial cell and intestinal organoid polarization in 3D using supramolecular hydrogels.

Methodology:
UPy supramolecular hydrogels were prepared in a fixed molecular ratio of 80 M to 1 B molecules at the desired concentration (wt%). For kidney cell studies in 3D, 0.5 mM of RGD UPy was incorporated into the hydrogel as part of the M molecules. Rheological experiments were measured at 1 rad/s and 1% strain.

Results:
Rheology showed that the mechanical properties of the UPy hydrogels could be varied from ~ 0.1 kPa to 1 kPa to 2 kPa for the 0.6, 1.25 and 2.5 wt% gels, respectively.
Madin-Darby Canine Kidney (MDCKs) were then encapsulated in 3D in UPy hydrogels of different weight percentages and corresponding stiffness. After 10 days, cell encapsulation resulted in cyst formation with apical-basal polarization inside all supramolecular hydrogels. Three different structures were formed, ranging from cell aggregates with inverted polarity to well-polarized cysts with a lumen inside. Quantification of the frequencies of the different morphologies in the different conditions showed that cell aggregates were predominantly formed in the 0.6 wt% gels, whereas the cysts with hollow lumens were mostly observed in the 2.5 wt% gels. This observation indicates that the cell-cell interactions dominate when less hydrogel, and thus less

mechanical support, is present.

Conclusions:
We showed that the cell-cell interactions dominate in the 0.6 wt% gels, whereas well-polarized cysts are formed in the higher, 2.5 wt% hydrogels. To further investigate the origin of the difference in organization, we will test the influence of ligand concentration. Currently, we are also investigating the influence of mechanical support (i.e. the wt% of UPy gels) on growth and polarity of intestinal organoids.

keywords: Supramolecular hydrogels, organoid culture, apical-basal polarity

41883605555

BILE DUCT ON A CHIP: ENGINEERING A MICROFLUIDIC PLATFORM FOR STUDYING BILIARY EPITHELIUM IN A DISH

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Background and Aims:
Biliary complications that may arise after liver transplantation, such as non-anastomotic strictures and diffuse bile leakage, are challenging and complex. Ischemia-related cell death and impaired regeneration of damaged biliary epithelium is known to be involved in causing these complications. Intrahepatic cholangiocyte organoids (ICO) allow for the expansion and study of cholangiocyte-like cells, but access to the lumen of the organoids is limited and can only be studied after disrupting the 3D structure. There are currently no in vitro models mimicking the circumstances as exposure of bile ducts to warm ischemia time or cold storage, and therefore we aimed to establish a microfluidic bile-duct-on-chip (BDOC) platform for studying the effect of these conditions on biliary epithelium in vitro

Method:
ICO were initiated from human liver biopsies (N=5) obtained during liver transplant procedures. Three-channel BDOC (dimensions; length 1cm, width and height 500µm) were prepared by casting polydimethylsiloxane (PDMS) into a mold. Subsequently, plasma treated PDMS chips were bonded to glass slides. The BDOC channels were filled with solubilized decellularized human liver extracellular matrix and collagen type I pre-gel. Viscous finger patterning procedures were used to create channels inside these hydrogels. ICO-derived cells (50 103 cells/channel) were introduced into the channels and ICO expansion medium was added to the reservoirs. The BDOC were incubated for up to 21 days. Growth of epithelial cells was monitored using confocal microscopy and histology.

Results:
ICO-derived cells populated the entire surface of the channels with a single layer of cells within 7 days after seeding. Whole mount confocal imaging revealed that cells were columnar in shape and morphologically looked like biliary epithelium. Zonula Occludens-1 (ZO-1) staining showed cholangiocyte-like polarization of cells in honey comb patterns. The cells express the cholangiocyte markers cytokeratin 7 and 19 on gene and protein level. Moreover, presence of glycocalyx components, such as fucosyl and sialic was confirmed based on histochemistry. This indicates that ICO form functional barriers.

Conclusion:
The results show that microfluidic approaches combined with cholangiocyte-like (KRT 7 and 19-positive) cells from ICO can be used to create healthy small diameter intrahepatic bile duct structures in vitro. This BDOC can thus serve as a platform to study epithelial damage and

regeneration during warm and cold ischemic conditions in more detail in vitro. The platform can also be used to study the onset and progression of biliary diseases.

keywords: Bile duct on chip, Intrahepatic cholangiocyte organoid, liver extracellular matrix, In vitro model

31412754957

DIFFERENTIALLY EXPRESSED MICRORNAS DURING ENDOCHONDRAL DIFFERENTIATION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS TO IDENTIFY POSSIBLE BIOMARKERS FOR NON-UNION FRACTURES

Franziska Breulmann (AO Research Institute Davos, Davos Platz, Switzerland), Manuel Herzog (AO Research Institute Davos, Davos Platz, Switzerland), Martin Stoddart (AO Research Institute Davos, Davos Platz, Switzerland), Elena Della Bella (AO Research Institute Davos, Davos Platz, Switzerland)

Possible biomarkers to predict the risk of healing delays are of huge clinical interest since 10% of fracture patients progress to delayed or non-union of fractures [1]. During endochondral ossification, which takes place in mechanically unstable regions with higher risk for delayed fracture healing, the bone regenerates through chondrogenic differentiation leading to a cartilage tissue before being remodeled into bone.
MicroRNAs (miRNA) are small, non-coding RNAs known to be involved in cell regulation [2] and play potential role in differentiation of human bone marrow derived mesenchymal stromal cells (hBM-MSCs). This work aims to identify miRNA differentially expressed during early differentiation of hBM-MSCs to be used as predictive biomarkers for non-union fracture healing.
hBM-MSCs were seeded and cultured for 28 days either in chondropermissive medium (CP) as negative control (DMEM 4.5 g/l glucose, 1% Pen/Strep, 1% ITS-x, 1% non-essential amino acids, 50 µg/ml ascorbic acid, 100 nM dexamethasone) or in chondrogenic medium (CH) as positive control (CP + 10 ng/ml TGF-β1) (N=4). Medium samples and pellets were collected on day 3, 7, 14, 21 and 28. Next, a multiaxial loading bioreactor is used for mechanically induced chondrogenesis of hBM-MSCs seeded in fibrin-polyurethane scaffolds, and three groups are compared (N=4): 1) scaffolds cultured in CP-medium (negative control), 2) scaffolds cultured in CH-medium (positive control), 3) scaffolds cultured in CP-medium + mechanical loading. To validate the differentiation of MSCs under chondrogenic conditions, histology with Safranin O/Fast Green staining was performed, as well as TGF-β1 ELISA and GAG analysis on medium and scaffold samples.
RNA was isolated from the pellets and scaffolds, followed by RT-qPCR to quantify miRNA and gene expression of chondrogenic marker genes, such as ACAN, SOX9, and COL2A1 during differentiation. As investigated in previous studies, miR-193a-5p is regulated chondrogenic differentiation of hBM-MSCs [3] and miR-193a-5p can be involved in regulation of TGF-β pathway [4]. We hypothesized that this miRNA has a regulatory role in chondrogenic differentiation, driven by TGF-β1 or during mechanically induced chondrogenesis.
Results miR-193a-5p are significantly downregulated in the scaffolds and appears downregulated in pellets cultured under chondrogenic conditions, confirming previous results [3]. Levels of miR-193a-5p during mechanically-induced chondrogenesis more closely resemble those in CP conditions. Since it has been reported that miR-193a-5p can be involved in bone metabolism by inhibiting the TGF-β pathway [4], we are investigating the correlation between levels of active TGF-β1 produced by mechanical stimulation with the miRNA expression at early days of differentiation processes. If there is a direct correlation, this miRNA may function as predictive markers for MSC differentiation and can be further validated for its role during endochondral ossification.

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keywords: microRNA, fracture healing, endochondral ossification, mechanically induced chondrogenesis, mesenchymal stromal cells

S57
Supramolecular synthetic scaffolds: from concept to design and application
Room: S2
(30 Jun 2022, 13:30 - 15:00)

Conveners: Saurav Ranjan Mohapatra,
Dammy Olayanju, Alberto Saiani

41935605049

SUPRAMOLECULAR BIOMATERIALS FOR ENGINEERING THE CELL-MATERIAL INTERFACE – FROM DESIGN TO SCREENING

Patricia Dankers (Eindhoven University of Technology, Eindhoven, The Netherlands)

The natural extracellular matrix (ECM) is a highly dynamic, supramolecular structure composed of various bioactive molecules held together by specific interactions. The ECM directly interacts with cells and dictates cell behavior to a large extend. Our goal is to synthetically mimic this intricate natural system using supramolecular materials based on hydrogen bonding units. The dynamics of the supramolecular system is shown to be important in the presentation of bioactive epitopes to cells. By design, highly dynamic supramolecular fibrous assemblies decorated with cell adhesive peptide motifs were made and studied in solution. It was shown that these soluble fibrous structures interact with the cell surface, and that the dynamics of bioactive presentation is dependent on the method of supramolecular incorporation. Transient networks and hydrogels composed of similar molecules were shown to have slowed down dynamics, compared to the particles in solution. These hydrogels, when formulated in the right way, were able to enhance cell viability and adhesion. When highly robust solid materials were made using the same supramolecular motif, cell adhesion and migration could be tuned. However, the ECM displays a plethora of bioactive peptide signals. Therefore, a high throughput screening approach was taken using a design of experiments set up, to investigate a synthetic library of peptides supramolecularly incorporated as additives in the base material. It was found that several sequences and/or combinations outperformed others, showing the importance of the high throughput screening approach. Our proposal is that both the dynamics and presentation of bioactive sequences determine cell behavior. In this way we aim to make steps towards the design of a synthetic ECM analogue.

keywords: supramolecular biomaterials, extracellular matrix, hydrogels, screening, molecular design

31451707839

NOVEL INSIGHTS INTO THE ORIGIN OF MY-FIBROBLASTS USING IPSC DERIVED KIDNEY ORGANOIDS MAINTAINED IN USER DEFINED SELF-ASSEMBLING PEPTIDE HYDROGELS

John Crean (University College Dublin, Dublin, Ireland)

Human induced pluripotent stem cell (hiPSC)-derived kidney organoids have prospective applications ranging from basic disease modelling to personalised medicine, however, there remains a requirement to refine the biophysical and biochemical parameters that govern kidney organoid formation. Here we describe the differention and maturation of hiPSC-derived kidney organoids within fully synthetic self-assembling peptide hydrogels (SAPHs) of variable stiffness (storage modulus, G′). The resulting organoids contained complex structures comparable to those differentiated within the animal-derived matrix, Matrigel. Single-cell RNA sequencing (scRNA-seq) was then used to compare organoids matured within SAPHs to those grown within Matrigel or at the air-liquid interface. A total of 13,179 cells were analysed, revealing 14 distinct clusters. Notably, differentiation within a higher G' SAPH generated podocytes with more mature gene expression profiles. Additionally, maturation within a 3D microenvironment significantly reduced the derivation of off-target cell types, which are a known limitation of current kidney organoid protocols. Finally, we show that these organoids can be used to faithfully model pathogenic processes; by integrating single cell gene expression and epigenome profiling, we identified de novo ACTA2+ve /POSTN+ve cell clusters in kidney organoids treated with TGFbeta, characterised by increased SMAD3-dependent cis chromatin accessibility and the expression of several genes associated with fibroblast activation in patients with Diabetic Kidney Disease. This work demonstrates the utility of synthetic peptide-based hydrogels with a defined stiffness, as a minimally complex microenvironment for the modelling of renal fibrosis.

keywords: Kidney Organoid, Hydrogel, Single Cell Multiomics

83767209244

IMPROVED GUANOSINE-BASED BIOINKS FOR SOFT TISSUE RECONSTRUCTIONS

Maria Godoy Gallardo (Internacional University of Catalonia, Sant Cugat del Vallès, Spain), Maria Merino Gómez (Internacional University of Catalonia, Sant Cugat del Vallès, Spain), Miguel Angel Mateos Timoneda (Internacional University of Catalonia, Sant Cugat del Vallès, Spain), F. Javier Gil (Internacional University of Catalonia, Sant Cugat del Vallès, Spain), Roman Perez Antoñanzas (Internacional University of Catalonia, Sant Cugat del Vallès, Spain)

Introduction
Patients requiring soft tissue reconstruction caused by defects or pathology may require biomaterials that provide a void volume for subsequent vascularization and new tissue formation, as autografts are not always a viable option. Here supramolecular hydrogels represent promising candidates due to their 3D structure being similar to the native extracellular matrix and their cell entrapment capability. Over recent years, guanosine (Guo)-based hydrogels have increasingly emerged, in which the nucleoside self-assembles into ordered structures (G4-quadruplex) and ultimately into nanofibrillar networks by the π-π stacking of G-quartets and coordination of central K+ ions. While such hydrogels typically exhibit a short lifetime, the use of boronic acid (BA) significantly enhances their stability. The aim of the present study was to combine this technology with 3D bioprinting to produce binary cell-laden hydrogels consisting of Guo and guanosine 5-monophosphate (GMP) stabilized by BA and K+, and to optimize printability and the survival of the entrapped cells. Such a system would then allow to tailor the biomaterial to the respective soft tissue defect and thus improve tissue reconstruction.

Experimental Methods
Various compositions of Guo (10-120 mM), GMP (10-120 mM), BA (10-60 mM) and KOH (10-60 mM) were mixed at 80 °C, slowly cooled down, and assessed for gelation by inversion test. Printability was subsequently evaluated by a semi-quantitative filament collapse and fusion test. The very best hydrogel composition was then immersed in a hyperbranched poly(ethylenimine) (PEI) solution (5 mgmL-1, 15min) and characterized by scanning electron microscopy (SEM) and rheological studies (strain sweep, dynamic step-strain sweep and peak-hold assay). Furthermore, nutrient permeability (FITC-Dextran) and hydrogel stability (immersion in complete medium at 37 °C) were determined. Finally, rat mesenchymal cells (rMCs) were entrapped in the hydrogels and studied for 21 days after printing, including cell viability and morphology assessment by confocal laser scanning microscopy (CLSM), and monitoring of the adipogenic differentiation (Oil-red O solution).

Results
From 49 hydrogel compositions that passed the inversion test, 15 exhibited suitable 3D printing properties. To improve long-term stability, hydrogels were subsequently treated with PEI, and the best composition was analyzed by SEM, showing nanofibrillar structures evident of successful G4-quadruplex formation. Furthermore, rheological analysis revealed good printing and thixotropic properties, while successful diffusion of FITC-dextran molecules (70, 500 and 2000 kDa) into the hydrogel confirmed that nutrients of various sizes may diffuse through the scaffold. Finally, a cell viability of 85% after 21 days was observed but with exclusively rounded morphology. However, lipid droplets were identified after 7 days, indicating cell functionality and successful differentiation under adipogenic conditions.

Conclusions

Our results demonstrate that printed Guo/GMP hydrogels exhibit extensive nanofibrillar networks and good printability and thixotropic properties. Stability was verified for 21 days in medium, and embedded cells showed good survival despite rounded morphology. Under adipogenic conditions, lipid droplets were observed, witnessing successful differentiation and functionality of the entrapped cells. Due to the demonstrated bioprintability, our Guo/GMP hydrogels may hold great potential for the reconstruction of soft tissues.

keywords: nucleoside-based hydrogels, guanosine and derivatives, 3D-bioprinting, cell-laden hydrogels, printable hydrogels

41883648366

WHERE ARE ALL THE ELECTROSPUN MEDICAL DEVICES? – CASE STUDIES OF PRODUCT DEVELOPMENT FROM AN INDUSTRY PERSPECTIVE

John Duckworth (The Electrospinning Company Ltd, Oxford, United Kingdom)

After decades of cutting-edge academic work, novel electrospun biomaterials are finally starting to enter clinics and patients worldwide. The process of taking an electrospun material from the lab, to the clinic, and finally to market is long and difficult. It requires the collaboration of many professionals over the course of years. But what it creates are world-class, ground-breaking medical devices which treat previously untreatable conditions, and improve the lives of patients.

This presentation will give a thorough overview of those devices containing electrospun materials which are currently available on the market, as well as those in various stages of clinical trials. We will explain how we, The Electrospinning Company Ltd, work with our clients to take exciting concepts born out of academia and private research, and guide them through proof-of-concept, design development, design control, production development and finally production. This presentation will also explain how pre-clinical and clinical trials fit into our framework, and how this all culminates in a final product release.

At The Electrospinning Company we can tailor our materials to achieve bespoke characteristics suitable for specific therapeutic indications and target tissues. By using nano and micro fibre structures, we can tightly control mechanical strength, flexibility, resorption time and architecture. Critically, we focus on producing these materials on an industrial scale, with the methodology, consistency, and quality necessary for medical applications. We refine ideas into standardised, repeatable processes to produce materials which can confidently be implanted into people.

Additionally, this presentation will give a detailed account of how The Electrospinning Company can take promising ideas and turn them into profitable products. Case studies of advanced materials developed for ophthalmological, dura mater repair, musculoskeletal, and cardiovascular applications will illustrate the process, showing how our scientists and engineers overcome one challenge after another on the long road to client and regulatory approval. The case studies will focus on specific technical and scientific challenges that had to be overcome, for devices in the clinic or about to enter the clinic.

keywords: electrospinning, scaffold, biomaterial, industry, resorbable

20941815547

DEVELOPMENT OF MULTIFUNCTIONAL ANTIMICROBIAL SUPRAMOLECULAR BIOMATERIALS

Martijn Riool (Amsterdam UMC, Amsterdam, Netherlands), Moniek Schmitz (Eindhoven University of Technology, Eindhoven, Netherlands), Leonie De Boer (Amsterdam UMC, Amsterdam, Netherlands), Patricia Dankers (Eindhoven University of Technology, Eindhoven, Netherlands), Sebastian Zaat (Amsterdam UMC, Amsterdam, Netherlands)

INTRODUCTION
The use of biomaterials inside the body always entails the risk of infection. This risk might even be higher in in situ tissue engineering applications. Since the porous scaffold materials can form a niche for invading bacteria, the intended in situ production of novel tissue may be severely compromised by infection. Therefore, we aim to develop a new polymeric supramolecular scaffold material, exerting two important functions: preventing microbial adhesion and thereby preventing biofilm formation, and inducing endogenous (eukaryotic) cells to regenerate the body.

METHODOLOGY
In our research, supramolecular contact-killing materials based on antimicrobial peptides (AMP) are developed. A special class of supramolecular biomaterials are based on fourfold hydrogen bonding 2-ureido-4[1H]-pyrimidinone (UPy) moieties. The supramolecular base material consists of an UPy end-functionalization polycaprolactone (i.e. PCLdiUPy). These UPy-materials can be functionalized with bioactive compounds, either via a modular approach in which the UPy-base material is mixed with UPy-modified additives1, or via a post-modification strategy to specifically functionalize the surface of the biomaterial using click chemistry2. The antimicrobial activity is introduced via UPy-functionalized AMPs, using SAAP-148, a synthetic derivative of LL-373. The regenerative activity is introduced via an UPy-functionalized heparin binding peptide (UPy-HBP). The peptides were synthesized by manual Fmoc-based solid phase peptide synthesis. Solid polymer films were prepared by drop-casting PCLdiUPy with UPy-SAAP-148 or UPy-TC84 on glass coverslips. The antimicrobial activity of the UPy-AMPs in solution and when incorporated in the drop-casted samples was evaluated against Escherichia coli ESBL and Staphylococcus aureus JAR060131 and LUH14616 (MRSA) and Acinetobacter baumannii RUH875 using the LC99.9 (i.e. the lowest concentration killing at least 99.9% of the inoculum) and the JISZ2801 surface antimicrobial assay, respectively. Moreover, the cytotoxicity of these AMPs was tested against human dermal fibroblasts.

RESULTS
Coupling of the UPy-linker to SAAP-148 did not influence its antimicrobial activity in solution. For the solid drop-casted materials, incorporation of 5 mol% UPy-SAAP-148 is sufficient for killing all 4 bacterial strains tested. This indicates that the peptide remains active after immobilization in the materials. Unfortunately, TC84 loses its antimicrobial activity upon UPy-coupling, both in solution and as a solid. QCM-D adsorption studies revealed that heparin adsorbed to spin coated material films of PCLdiUPy with 5 mol% UPy-HBP mixed via the modular strategy. Current studies focus on characterization of the UPy-SAAP-148/TC84 and multifunctional biomaterial with XPS, AFM, WCA, zeta potential and leakage experiments to investigate the material properties. Moreover, we assess the in vivo efficacy of dip-coated titanium implants with 5% UPy-SAAP-148 in the experimental biomaterial-associated infection mouse model.

CONCLUSIONS
In conclusion, this modular approach will enable a stable but dynamic incorporation of AMPs, and control of cell adhesion by using cell-adhesive peptides. Ultimately, we aim to use such materials for in situ infection-free tissue engineering.

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keywords: supramolecular materials; antimicrobial peptides, infection, biomaterial, in vivo

52354517559

TISSUE ENGINEERING THE OESOPHAGUS: PROOF-OF-CONCEPT

Nischal Rai (University of Manchester, Manchester, United Kingdom), Julie Gough (University of Manchester, Manchester, United Kingdom), Alberto Saiani (University of Manchester, Manchester, United Kingdom), Sarah Herrick (University of Manchester, Manchester, United Kingdom)

INTRODUCTION
Current treatments for oesophageal cancer and oesophageal atresia, that involves the repair of the entire thickness of the oesophagus, present various complications and challenges due to the lack of functional oesophageal replacement tissue. Through the combination of cells, scaffolds and biologically active molecules, tissue engineering presents an innovative approach to develop constructs that can mimic the multi-layered architecture of the oesophagus. This study analyses the response of primary oesophageal epithelial cells and fibroblasts on Manchester BIOGEL's various self-assembling peptide hydrogels (PeptiGels), and primary oesophageal smooth muscle cells' response on hydrogel-coated polycaprolactone (PCL) scaffolds with aligned fibres to determine a suitable hydrogel and PCL scaffold combination to tissue engineer a simplified oesophagus, consisting of epithelial, submucosal and muscle layers.

METHODOLOGY
2D culture of human oesophageal epithelial cells on PeptiGels was performed using a cell density of 3,000 cells/mm2; and cell viability assay, metabolic assay and immunohistochemical analysis were performed up to day 21. 3D culture of human oesophageal fibroblasts within PeptiGels was performed using a cell density of 100,000 cells/100μL volume of hydrogel; and cell viability assay, proliferation assay and immunohistochemical analysis were performed up to day 21. Aligned electrospun PCL fibres were coated with PeptiGel Alpha4_RGD_GFOGER before human oesophageal smooth muscle cells were seeded onto the PeptiGel-coated PCL scaffolds at 40,000 cells/cm2. Cell viability assay, metabolic assay and immunochemical analysis were performed up to day 21.

RESULTS
Viable cells, increase in metabolic activity and increased cell proliferation at greater timepoints were recorded for epithelial cells cultured in 2D and fibroblasts cultured in 3D for all PeptiGels up to day 21. Immunohistoanalysis showed positive expression of ZO-1 tight junction protein and involucrin markers by epithelial cells seeded on all PeptiGels up to day 14, and positive expression of Collagen I and Collagen III proteins, and Keratinocyte Growth Factor by fibroblasts seeded into all PeptiGels up to day 14. Analysis of results at this stage indicated that a positively charged hydrogel with RGD and GFOGER motifs, i.e., PeptiGel Alpha4_RGD_GFOGER, to the be most suitable out of all hydrogels for 2D and 3D culture of epithelial cells and fibroblasts respectively. PeptiGel Alpha4_RGD_GFOGER was chosen to pre-coat aligned PCL fibres for smooth muscle cell culture.
Viable cells and increase in metabolic activity at greater timepoints were recorded for smooth muscle cells cultured on PeptiGel-coated PCL scaffolds up to day 21 and positive expression of smooth muscle alpha-actin marker by smooth muscle cells seeded on PeptiGel-coated PCL scaffolds were recorded up to day 21.

CONCLUSIONS
The results collectively indicate that employing a combination of synthetic peptide hydrogels and hydrogel-coated aligned PCL fibres to tissue engineer the multi-layered structure of the oesophageal tissue is a viable option.

keywords: Tissue Engineering, Oesophagus, Hydrogels, PCL, Biomaterials,

20941807266

DESIGN OF 3D PRINTABLE SUPRAMOLECULAR SELF-ASSEMBLING Β-SHEET PEPTIDE-HYALURONIC ACID HYDROGELS WITH IMMUNOMODULATORY PROPERTIES

Jacek K. Wychowaniec (AO Research Institute Davos, Davos, Switzerland), Ezgi Irem Bektas (AO Research Institute Davos, Davos, Switzerland), Nicolas Devantay (AO Research Institute Davos, Davos, Switzerland), David Eglin (Mines Saint-Étienne, Univ Lyon, Univ Jean Monnet, INSERM, U1059 Sainbiose, Saint-Étienne, France), Matteo D'Este (AO Research Institute Davos, Davos, Switzerland)

Introduction
Delayed or severed tissue regeneration is often caused by dysfunctional immune system.[1] One solution to tackle this issue is to design immunomodulatory materials that support tissue regeneration by priming immune system to a pro-regenerative state.[2] For example, it is known that high molecular weight (>1000 kDa) hyaluronic acid (HA) can polarize macrophages to an M2 pro-regenerative phenotype, whereas low molecular weight HA drives pro-inflammatory M1 polarization.[3] Understanding the rules for designing functional materials that incorporate immunomodulatory effects, biocompatibility and allow stable long-term polarization of macrophages is therefore of high interest in multiple tissue engineering (TE) scenarios, notably for 3D printing TE.

Methodology
Here, to answer the new demands, we designed a selection of two-component hydrogels built from self-assembling β-sheet forming peptides[4] and immunomodulatory tyramine-modified HA (THA)[5], that can be processed by 3D micro-extrusion printing. A selection of peptide sequences was based on the alternation of hydrophobic and hydrophilic amino acids: XYXZXYXZ (X: hydrophobic residue: phenylalanine or tyrosine, Y/Z: hydrophilic residue e.g.: lysine or glutamic acid), stemming from the known parental FEFKFEFK sequence and it subsequent modifications.[4] All parental peptides self-assemble into semi-flexible networks and hydrogels, as derived from oscillatory rheology measurements and contain high β-sheet content, as measured by FTIR. 280 kDa and 1640 kDa THA were synthesized as previously described.[4] The successful THA synthesis was confirmed using 1H-NMR and degree of modification was calculated from UV absorption.

Results
A parametric study was carried out to verify the effect of rational peptide sequence modification on final physico-chemical and biological properties of composite hydrogels. Self-assembly, and rheological properties can be controlled by the choice of primary peptide sequence, fabrication technique and final crosslinking mechanisms including enzymatic (HRP, H_2O_2) and visible green light crosslinking using Eosin. These hydrogels are characterised by shear-thinning behaviour and rapid recovery allowing extrusion-based fabrication of both simple (lines, grids) and more complex shapes retaining post-printing fidelity.

Conclusions
The versatile crosslinking mechanisms allow post-crosslinking structure stabilization with longer-term degradation, deeming them a modular and versatile inks platform to endow with multiple biological cues for TE and immunomodulation.

ACKNOWLEDGEMENTS:
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keywords: hyaluronic acid, peptides, hydrogels, immunomodulation, 3D printing

S58
TERMIS-EU SYIS and yESAO joint symposium
Room: S4 C
(30 Jun 2022, 15:30 - 17:00)

Conveners: Lisanne Laaglan, Zuzana Koci,
Yi-Tung Lu

52354524437

NANOENGINEERED MECHANICALLY ROBUST BIOACTIVE PARTICLES DISSEMINATED IN CHITOSAN/COLLAGEN MATRIX FOR OSTEOPOROTIC BONE TREATMENT

Kulwinder Kaur (Royal College of Surgeons in Ireland, Dublin, Ireland), Ciara Murphy (Royal College of Surgeons in Ireland, Dublin, Ireland)

Introduction
Osteoporosis, characterised by depleted bone mass and disrupted bone architecture due to impaired bone remodelling, is the most prevalent metabolic bone disease in the world, causing fractures worldwide at a rate of one every 3 seconds, exceeding health care costs of € 37 billion each year1. Osteoporotic vertebral fractures (OVFs) are the most common complication of osteoporosis and patients determined to have OVFs are 5 times more likely to suffer secondary vertebral fragility fractures2. The clinical gold standard of care for OVFs is vertebroplasty and kyphoplasty, whereby cement is injected into the damaged vertebrae to stabilise the fracture site and reduce pain. These cements are not biodegradable and often leading to complications such as cement leakage and appearance of secondary fractures in adjacent diseased vertebrae1. So, the main aim of this study was to tackle a devastating clinical orthopaedic challenge for which there is currently no reparative treatment specifically OVFs, by developing an advanced mechanically robust biomaterial technology to repair & restore structural integrity and function of disease damaged bone. These materials can be loaded with different agents to promote a targeted delivery, reducing the occurrence of side effects common in conventional treatments.

Methodology
Strontium loaded nano hydroxyl apatite particles (nHAS) functionalized with SWCNTs were prepared by using wet-precipitation method. Thermoresponsive nHAS decorated hydrogels were prepared by using β-GP3 and scaffolds were prepared by freeze dried method4. Physicochemical properties was assessed by using XRD, FTIR, SEM, TGA, DSC,TEM techniques ,degradation profile in PBS and mechanical properties by Zwick Roell testing machine using 5N load. Rat mesenchymal stem cells were seeded on the scaffolds to assess osteogenesis activity via cytotoxicity, proliferation and quantitative RT-PCR to detect key osteogenic markers, ALP activity and calcium deposition. RAW 267.4 cells were used to check the osteoclastogenesis effect of releasing Sr ion via TRAP activity and RT-PCR to detect key osteoclastogenic markers.

Results
Mechanically robust scaffolds for controlled degradation and ion releasing profile were prepared. All the scaffolds was found to be have high water retention ability, porous and bioactive in nature. Scaffolds are found to be non-toxic with enhanced osteogenic differentiation and promote mineralised matrix deposition with increasing content of Sr, and decreased TRAP activity for RAW 264.7. This shows the repair & restore structural integrity of our scaffolds for disease damaged bone without the need of added additional therapeutics.

Conclusion
We developed a therapeutic mechanically robust biomaterial technology that will for the first time, combine mechanically robust carbon nanotubes with antiosteoclastic-ion substituted nano-particles, to target impaired bone remodelling and drive regeneration in a disease compromised load-bearing environment.

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comment: The author acknowledges Irish Research Council, Government of Ireland Post-Doctoral Fellowship (GOIPD/2019/793) for funding. Collagen materials were provided by Integra Life Sciences, Inc. through a Material Transfer Agreement. Royal College of Surgeons for providing facilities for this work is highly acknowledged. There is no conflict of Interest.

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keywords: Nanoengineered hydrogels, Osteoporotic, Mechanically robust, Hydroxyapatite, Scaffolds

52354545528

THE DIFFERENTIAL RESPONSE OF HUMAN MACROPHAGES TO 3D PRINTED TITANIUM ANTIBACTERIAL IMPLANTS DOES NOT AFFECT THE OSTEOGENIC DIFFERENTIATION OF HMSCS

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Introduction
Despite considerable developments in the field of orthopedic implants, complications including poor bone ingrowth and implant-associated infections (IAI) persist. Macrophages have recently been acknowledged to be essential for the implant success in the body, partly through intimate crosstalk with mesenchymal stem cells (MSCs) in the process of new bone formation [1]. However, the behavior of these immune cells is known to be affected by environmental cues, including the implant surface properties. Additive manufacturing (AM), surface biofunctionalization, and silver nanoparticles incorporation are promising techniques to achieve orthopedic implants with osteogenic, immunomodulatory, and antibacterial biofunctionalities [2–4]. The osteoimmunomodulatory properties of such implants are, however, not yet well understood. We, therefore, investigated the effects of human macrophages on the human mesenchymal stem cells (hMSCs) when co-cultured in vitro with AM titanium implants biofunctionalized via plasma electrolytic oxidation (PEO) and incorporated with silver nanoparticles (AgNPs).

Methodology
AM generated Ti-6Al-4V implants were biofunctionalized via PEO with/without AgNPs. Surface characterization was performed with a scanning electron microscope (SEM) and silver ion release was measured. The effects of the incorporation of AgNPs at different concentrations on human macrophages and bacterial cells were assessed by evaluating the viability of human macrophages and performing an isothermal microcalorimetry assay where bacterial metabolic activity was measured. The response of human macrophages and hMSCs monocultures to the PEO-treated Ti-6Al-4V implants were subsequently evaluated by measuring mineralization, protein, and gene expression. Finally, an indirect co-culture of macrophage-hMSCs was performed to study the effects of the macrophage response induced by the implants on the hMSCs osteogenic differentiation.

Results
The PEO modification of the AM implants created TiO_2 surfaces with micro- and nano-porosities. AgNPs were successfully incorporated into the TiO_2 layer. A concentration of 0.3 g/L AgNPs was found to be optimal to maintain the viability of human macrophages while imparting sufficient antibacterial properties to prevent bacterial growth on their surfaces. The expression of tissue repair related factors decreased in the specimens containing 0.3 g/L AgNPs as compared to the PEO-treated specimens not incorporating AgNPs. The same trend was observed for the macrophages co-cultured with hMSCs. However, this did not affect the osteogenic differentiation of hMSCs. Both co- and single-cultured hMSCs could osteogenically differentiate without any adverse effects caused by the presence of macrophages that were exposed to the either surface.

Conclusions
Based on the findings of this study, the incorporation of AgNPs into the PEO layers does not compromise the osteogenic differentiation and mineralization of hMSCs when co-cultured with human macrophages, while adding antibacterial functionalities to AM surfaces. Further evaluation of these promising implants in a bony in vivo environment with and without infection is, thus, recommended, and may prove them worthy of further development for potential clinical use.

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[4] F. Razzi et al., Biomed Mater. 15, 035017 (2020)

keywords: Osteoimmunomodulation, Bone implants, Macrophages, Mesenchymal stem cells, Silver nanoparticles

S59+S18
The role of multifunctional nanomaterials in new tissue regeneration strategies + Biomedical applications of MXene based next generation nanomaterials
Room: S3 A
(30 Jun 2022, 13:30 - 15:00)

Conveners: Aleksandra Benko, Sanjiv Dhingra,
Lucia Gemma Delogu

83871202439

NANOMEDICINE: HAVING EXTERNAL CONTROL OF TISSUE ENGINEERED MATERIALS AFTER IMPLANTATION

Thomas Webster (Hebei University of Technology, Tanjian, China)

Nanotechnology is now found in almost every aspect in life, from the liposomes that carry COVID-19 vaccines to coatings placed on floors to reduce wear. Over the past 20 years, the use of nanotechnology in medicine has grown from the unknown to now significantly helping to prevent, diagnosis, and treat numerous diseases. This is true for regenerative medicine and tissue engineering as well. Specifically, nanotechnology has been used to create biologically-inspired nanotextures to promote tissue formation, limit infection, and decrease inflammation. It has also been used to create materials whose shape and function can be changed after being implanted into the body. Such materials can be used to straighten the curves of spines for patient suffering from scoliosis, close sphincters in the body such as from the stomach to intestines, increase pressure on juxtaposed bones to promote bone growth, control drug delivery, and so much more. This invited talk will dsicuss all of the promising applications of nanotechnology in tissue engineering, in particular emphasizing in vivo studies and what is needed for the incorporation of nanotechnology into tissue engineering to continue to grow and meet the high demands of medicine in the future.

keywords: nanomedicine, nanotechnology, tissue engineering, regenerative medicine

31451706027

THE ROLE OF MULTIFUNCTIONAL NANOMATERIALS IN NEW TISSUE REGENERATION STRATEGIES

Gwendolen Reilly (University of Sheffield, Sheffield, United Kingdom), Denata Syla (University of Sheffield, Sheffield, United Kingdom), Jose Oliveira Rodrigues (University of Sheffield, Sheffield, United Kingdom), Laura Grillini (Finceramica, Faenza, Italy), Lucia Forte (Finceramica, Faenza, Italy), Riccardo Bendoni (Finceramica, Faenza, Italy), Frederick Claeyssens (University of Sheffield, Sheffield, United Kingdom)

Synthetic hydroxyapatite is therapeutically used as bone graft substitute, bone filler, or as coatings to support attachment of bone to metal implants. Here I will present some data on how our group have used hydroxyapatite nanoparticles in combination with various polymers and fabrication techniques to support bone cell differentiation and matrix formation in both static and mechanically stimulated culture conditions. However, the slow degradation rate of hydroxyapatite compromises its osteogenic activities so recently we have collaborated with industrial partners to create a multisubstituted HAP (sHAP) with Magnesium and Strontium. We used a continuous flow method and a Design of Experiments approach to optimise the method and amounts of magnesium and strontium in the hydroxyapatite to increase its solubility, osteogenic integrity and bioactivity. The powders were tested using immortalised human mesenchymal stem cells Y201 in serum-free media and shown to support osteogenesis with no cytotoxicity at any dose tested. A particular need for improved orthopaedic devices is in spine repair where 50% of spinal fusion surgeries need revision partly due to poor osseointegration. Therefore we employed our substituted hydroxyapatite in two spine repair devices 1) Spinal fusion cages: as a coating on a titanium oxide base on a PEEK spinal cage 2) Bone graft substitute: as a particulate phase within a sHAP polycaprolactone composite use as an ink to print an insert for spinal cages which can be used in place of bone graft. Materials containing sHAP were tested for their ability to support osteogenesis and their potential to accelerate the fusion process in spine repair.

keywords: Bone, orthopeadic, spine, nano-coatings, composites

31412769528

CARBON NANOTUBES AS EFFECTIVE MODULATORS OF CELLULAR REACTIONS IN VARIOUS TISSUE REGENERATION STRATEGIES

Aleksandra Benko (AGH University of Science and Technology, Kraków, Poland), Michał Dziadek (Jagiellonian University, Faculty of Chemistry; AGH University of Science and Technology, Kraków, Poland), Joanna Duch (Jagiellonian University, Faculty of Chemistry, Kraków, Poland), David Medina-Cruz (Northeastern University, Boston, United States), Sebastian Wilk (AGH University of Science and Technology, Kraków, Poland), Katarzyna Cholewa-Kowalska (AGH University of Science and Technology, Kraków, Poland), Andrzej Kotarba (Jagiellonian University, Faculty of Chemistry, Kraków, Poland), Thomas Jay Webster (Interstellar Therapeutics, Boston, United States)

Introduction
Efficient regeneration of different tissue types requires solutions that are specifically tailored to meet certain criteria. This is particularly true for the group of hard-to-regenerate tissues, such as cardiac, neural or chondral, which are known to have a low self-regeneration potential. The cells of these tissues often require certain factors to induce their division, differentiation and maturation. These would include usage of scaffolds with: specific surface properties (chemical composition, presence of certain motifs), presence of some sort of bioactive compound (various types of biomolecules), electro-donor properties, electrical conductivity, surface morphology, mechanical properties [1, 2]. Positive therapeutic effect can be further boosted by introducing additional exogenous stimuli, such as electrical, magnetic, or mechanical stimulation [3, 4]. Certainly, all of the positive outcomes can be boosted when all of the above-mentioned cues are combined.
Despite controversies regarding their safe biomedical applications, carbon nanotubes (CNTs) of well- defined properties have been proven be biocompatible, creating interesting modificators for the fabrication of multifunctional scaffolds with new or improved properties. Electrical conductivity, ability to bind and controllably release bioactive compounds, or introducing the stimuli-responsiveness are just some examples of these properties.

Methodology
In this study, surface functionalization of CNTs have been employed to grant them with cytocompatibility and bioactive properties. Next, the CNTs were used as matrix and surface modifiers. Chemical composition, electrical and mechanical properties of the as-obtained scaffolds were evaluated and the materials were tested for their effect on cells, antibacterial, and anticancer properties.

Results & conclusion
In the course of this study, electrically conductive and cytocompatible materials based on CNTs were fabricated. Altered electro-donor, physichochemical and electrical properties yielded surfaces of bactericidal and anti-cancer properties that at the same time were able to enhance the cellular adhesion, growth and proliferation.

Acknowledgements
This study was supported by the National Science Centre, Poland, under grants nos. UMO-2017/24/C/ST8/00400 and 2020/37/B/ST5/03451.

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keywords: carbon nanotubes, multifunctional biomaterials, electroconductive biomaterials, antibacterial properties

31412719397

SPATIALLY RESOLVED MONITORING OF IN VITRO AND IN VIVO DEGRADATION IN CARDIOVASCULAR IN SITU TISSUE ENGINEERING

Julia Marzi (Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Tübingen, Germany), Emma Munnig Schmidt (Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands), Eva Maria Brauchle (Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Tübingen, Germany), Tamar Wissing (Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands), Hannah Bauer (Xeltis BV, Eindhoven, Netherlands), Martijn A.J. Cox (Xeltis BV, Eindhoven, Netherlands), Anthal I.P.M. Smits (Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands), Katja Schenke-Layland (Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Tübingen, Germany)

Introduction
In situ tissue-engineered vascular grafts (TEVGs) based on resorbable synthetic scaffolds have the potential to overcome the limitations of prosthetic graft replacement and provide off-the-shelf availability. Despite massive efforts in investigating new materials, up to date no TEVG is clinically available. One of the most important challenges for successful regeneration is to balance the scaffold degradation and tissue formation. There is an increasing demand for tools to monitor these parameters simultaneously and at a spatial resolution, as most of the currently applied methods can either access polymer degradation or tissue regeneration.

Methodology
Raman microspectroscopy allows to characterize a sample based on molecule-specific spectral fingerprints, which enables label-free evaluation and imaging of a sample. This study investigated the potential of Raman microspectroscopy as an in situ tool to monitor degradation kinetics and mechanisms of supramolecular polymers which are applied as degradable scaffolds in in situ tissue engineering. Raman imaging was applied on in vitro degraded polymers, investigating different polymer materials, subjected to oxidative and enzymatically induced degradation. Furthermore, the method was transferred to analyze in vivo degradation of tissue-engineered carotid grafts after 6 and 12 months in a sheep model.

Results
Multivariate data analysis allowed to trace degradation and to compare the data from in vitro and in vivo degradation, indicating similar molecular observations in spectral signatures between implants and oxidative in vitro degradation. In vivo degradation appeared to be dominated by oxidative pathways. Furthermore, collagen remodeling at the implant interface was monitored simultaneously to the assessment of polymer degradation.

Conclusion
Our results demonstrate the sensitivity of Raman microspectroscopy and imaging to determine degradation stages and the assigned molecular changes non-destructively, encouraging future exploration of this techniques as a quality assessment tool to monitor in situ tissue engineering.

keywords: in situ tissue engineering, Raman imaging, cardiovascular, degradation

41883625484

SMART TANTALUM CARBIDE MXENE QUANTUM DOTS FOR TREATMENT OF ALLOGRAFT VASCULOPATHY

Weiang Yan (Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, Canada), Alireza Rafieerad (Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, Canada), Keshav Narayan Alagarsamy (Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, Canada), Leena Regi Saleth (Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, Canada), Sanjiv Dhingra (Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, Canada)

Introduction
Allograft vasculopathy is an aggressive form of accelerated atherosclerosis that manifests uniquely in transplanted hearts, lungs, and kidneys. Activated blood vessel endothelial cells (ECs) stimulate alloreactive CD4+ and CD8+ T-lymphocytes to result in sustained inflammation. Transition metal carbides, MXenes, are an emerging class of nanomaterials that have recently been shown to have unique immunomodulatory properties. Here, we present the development and application of novel tantalum carbide MXene (Ta4C3Tx) quantum dots for in vivo immunomodulation and prevention of allograft vasculopathy.

Methodology
To infer mechanisms and to ensure reproducibility of results, detailed physicochemical characterization of Ta4C3Tx MXene quantum dots was performed using scanning/transmission electron microscopy, x-ray diffraction, and x-ray photoelectron spectroscopy. In vitro studies were carried out using co-cultures of human umbilical vein endothelial cells (HUVECs) with allogeneic peripheral blood mononuclear cells, and immunomodulatory function was assessed using flow cytometry. Mechanisms for immunomodulation was ascertained using quantitative real-time PCR. A rat aortic transplantation and allograft vasculopathy model was used for in vivo validation of safety and immunomodulatory function.

Results
A facile hydrofluoric acid-free protocol was rationally designed to synthesize a zero-dimensional MXene quantum dot (MQD) material. These MQDs were surface modified with high amounts of different functional groups. The average diameter of single particles was found to be about 3.5 nm. Using the in vitro co-culture system, we found that Ta4C3Tx MQDs interact with activated human ECs to reduce activation and pro-inflammatory Th1 polarization of allogeneic CD4+ lymphocytes. Furthermore, we showed that treatment with MQDs significantly increased endothelial surface expression of the T-cell co-inhibitory molecule PD-L1 and decreased expression of the costimulatory molecule CD86. Finally, when applied in vivo¸ our data suggested that treatment with MQDs could significantly reduce lymphocyte infiltration and preserve medial smooth muscle cell integrity within transplanted vessel segments.

Conclusion
These findings offer the promise of next generation Ta4C3Tx MQDs as a smart material for treatment of allograft vasculopathy and other inflammatory diseases. This research also opens the door to development of rationally designed Ta4C3Tx MXene quantum dot technologies for other immune-sensitive regenerative medicine applications.

keywords: MXene, Immunomodulation, Nanomaterials, Regenerative Medicine

41883634455

AEROSOL-JET PRINTING ENABLES HIGH-RESOLUTION TI3C2 MXENE PRINTED ELECTRODES ON A PTFE STRUCTURE FOR NEURAL STIMULATION

Javier Gutierrez Gonzalez (Trinity College Dublin, School of Chemistry, Dublin, Poland)

Introduction
Spinal cord injury (SCI) is a devastating condition that disrupts both sensory and motor function, with very limited prospects of functional recovery. Electrical stimulation (ES) has become a common clinical remedy to lessen the impact of SCI-induced pain after injury. However, regeneration-focused lesion site electrostimulation has not had clinical translation yet, despite promising evidence of directing axonal growth and encouraging cord repair. Furthermore, neural interfaces still face challenges over long implantation times due to delamination, insufficient water barrier properties and inflammatory responses. MXenes, a novel class of 2D electroconductive layered materials, possess unique properties for developing ES systems that can wrap around the injured cord to deliver charge safely and efficiently.
Here we show the aerosol jet 3D printing (AJP) of a neural interface cuff with highly conductive MXene (Ti3C2TX) electrodes, protected and insulated by a polytetrafluoroethylene (PTFE) structure.

Methodology
MXene films were produced using doctor blade, vacuum-assisted and AJP-printing to assess the effect of fabrication methods on their physical properties and biocompatibility. Conductivity, hydrophilicity and mechanical properties from the films were evaluated.
To assess biocompatibility, NSC-34 mouse motor neurons were seeded on the MXene films to study the morphology influence onto the cells over 3 days and their morphology, proliferation and metabolic rate were studied.
PTFE substrate was spin-coated with a commercial ink followed by 3D printing of the MXene circuit using an Optomec AJP-300 3D printing system. Then, the circuit was passivated and protected with another layer of PTFE by 3D printing with the same system, and then sintered at 360^0C under non-oxidizing argon atmosphere to create a solid structure.

Results
Results showed that all MXene films were biocompatible, supporting neuron cell viability via similar proliferation and metabolism rate to tissue culture polystyrene controls. Also, neurons grown on AJP-printed films displayed enhanced neuronal neurite outgrowth and cell morphology, possibly, due to their enhanced conductivity (12000 S/cm) and higher hydrophilicity (35^0) in comparison to filtered and doctor blade films. Top and cross-section SEM images of the device showed conformal deposition of MXenes onto a printed PTFE substrate, facilitated by the surfactant-induced hydrophilicity of the PTFE commercial ink. After sintering at 360^0C, the PTFE nanoparticles coalesced, effectively bounding the printed MXenes onto its otherwise hydrophobic surface. During the thermal treatment, the surfactant and remaining moisture from the PTFE ink evaporated, switching the behaviour of the PTFE surface from hydrophilic (41^0) to hydrophobic (125^0) ($p<0.0001$).

Conclusion
Direct adhesion of 3D printed MXenes after PTFE sintering postulates as a facile method to construct bespoke neural electrode implants for stimulation of the injured spinal cord, while limiting abiotic and biotic faults due to the excellent biocompatibility and pliability of the device.

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keywords: MXenes, Thin film, flexible electronics, electrostimulation, spinal cord injury

83767273539

THE IMPACT OF PRIMARY AND SECONDARY FIBERS MORPHOLOGY ON REGENERATIVE AND OPTICAL PROPERTIES OF ELECTROSPUN CORNEA IMPLANT

Roksana Kurpanik (Faculty of Materials Science and Ceramics, Department of Biomaterials and Composites, AGH University of Science and Technology, Krakow, Poland), Ewa Stodolak-Zych (Faculty of Materials Science and Ceramics, Department of Biomaterials and Composites, AGH University of Science and Technology, Krakow, Poland), Marcin Kudzin (Łukasiewicz Research Network – Textile Research Institute, Łodz, Poland), Anna Ścisłowska-Czarnecka (Department of Cosmetology, Academy of Physical Education, Krakow, Poland)

Introduction
Lack of a cornea's donor is still a huge problem in ophthalmology leaving over 98% of people waiting for the transplantation. As the solution, bioengineered scaffolds, mimicking the fibrous structure of the corneal stroma are proposed. To obtain a full regeneration, such substrates should provide both cornea cells repopulation and optical transparency. In the native cornea, the hierarchical microstructure of collagen fibrils is responsible for maintaining those properties. Studies have proved that both primary morphology (organization of the fibres) and secondary morphology (fibres nanotopography) have a huge impact on the phenotype of the cells.
In our study, we investigated the impact of different primary (aligned and random fibres) and secondary microstructure (shish-kebab, beads-on-strings) of the electrospun PCL nonwovens on the viability of different cells (macrophages, keratinocytes, fibroblasts) to check whether they show a preference for a specific type of surface. We also examined the correlation between the morphology of the substrate and the optical transparency of the scaffold.

Methodology
The nonwoven was obtained by the electrospinning method based on polycaprolactone (PCL). To obtain the aligned fibres the rotating drum collector was used. Coaxial electrospinning was used to prepare beads-on-strings fibres using different concentrations of PCL solution in a shell/core part. To obtain shish kebab morphology, the neat fibres were modified by directional crystallization in PCL solution. The morphology of the samples was observed under scanning electron microscopy. The influence of the obtained fibres morphology on the physicochemical properties of the membrane (wettability, surface energy) was also investigated. The light transmission through the scaffold was examined by UV-VIS analysis. A biocompatibility test was performed by contacting the cells with the fibrous scaffold after 3 and 7 days.

Results
The SEM observations showed the presence of the randomly oriented and aligned nano- and submicron fibres. All of the obtained fibres have unimodal size distribution in a range 400 – 1000nm. The directional crystallization enabled achieving fibres with lamellae parts, characteristic of the shish-kebab morphology. The use of coaxial electrospinning enabled to obtain bead-on-strings fibres with a reduced core diameter (in a range of 200 – 700nm). Independently on the modification all of the samples exhibited hydrophobic properties (contact angle ~130°). The research also showed a slight difference in translucency between random oriented and aligned fibres, however, the best light transmission was recorded for beads-on-strings fibres. All of the samples exhibited high viability of all cells types independently of the orientation nor morphology of the fibres. None of the tested scaffolds showed a cytotoxic effect.

Conclusion(s)
Electrospun nanofibers with different microstructures were successfully obtained and demonstrate biological and optical properties that indicate the strong potential as extracellular matrix – mimicking substrates for cornea regeneration.

Acknowledgement
This work was supported by the subsidy of the Ministry of Education and Science for the AGH University of Science and Technology in Kraków No 16.16.160.557.

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keywords: Electrospinning, beads-on-strings, shish kebab, random and aligned fibers, cornea

S60
Tissue engineering and regenerative medicine in Czech Republic
Room: S4 B
(30 Jun 2022, 11:00 - 12:30)

Conveners: Giancarlo Forte, Josef Jaros

94355105005

THE MOLECULAR BASIS OF PATHOLOGICAL MECHANOSENSING IN THE FAILING HEART

Giancarlo Forte (St. Anne's University Hospital, Brno, Czech Republic)

The onset and progression of aging-associated pathologies is paralleled by continuous local extracellular matrix (ECM) remodelling. This process serves as a compensatory strategy for tissues to cope with the altered conditions.

The modifications in the nanostructure and mechanics of cardiac ECM are driven by the activation of cardiac fibroblasts and impair cardiac cell function to progressively lead to organ failure. In turn, cardiomyocytes respond to the ensuing biomechanical stress by re-expressing fetal contractile proteins, by transcriptional and post-transcriptional processes, such as alternative splicing.

Our group demonstrated that the aberrant activation of mechanosensitive Yes Associated Protein (YAP) alters the assembly of focal adhesions in response to mechanical stress. Additionally, we contributed knowledge on YAP regulation during the acquisition of cardiac phenotype by adult and pluripotent stem cells, and found that its hyperactivation in patient-derived cardiac fibroblasts promotes ECM pathological remodelling, thus favoring the fibrotic process and fueling heart failure.

Lately our experimental data highlighted how the pathological remodeling of ECM in the failing heart directly affects the expression and function of RNA binding proteins in cardiomyocytes. This discovery demonstrated that mechanical stress can effectively rewire the alternative splicing of numerous genes involved in cardiomyocyte contractility, calcium handling and mechanosensing.

These studies allowed us to describe different layers of intracellular mechanosensing responsible for finely tuning the expression of splicing variants of important cardiac genes in response to pathological mechanical turmoil.

keywords: Czech TERMIS, mechanobiology, translational medicine, Hippo pathway

41883636846

UNVEILING THE MOLECULAR BASIS OF PATHOLOGICAL MECHANOSENSING TO COUNTERACT DISEASES

Vladimir Vinarsky (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Stefania Pagliari (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Soraia Fernandes (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Marco Cassani (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Pavel Simara (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Jorge Oliver De La Cruz (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic), Giancarlo Forte (International Clinical Research Center of St. Anne's University Hospital, Brno, Czech Republic)

Following ECM pathological remodelling, the aberrant activation of the mechanosensing apparatus contributes to the establishment and progression of age-related pathologies, like those affecting the cardiovascular system, and cancer. The mechanical turmoil associated with ECM remodeling is known to determine opposite consequences in rather different cell types, like cardiac and tumor cells: while cardiomyocytes in the heart are induced to hypertrophy by mechanical stress, cancer cells tend to proliferate and disseminate following similar stimuli.

Our group recently demonstrated how ECM pathological remodeling determines changes in the assembly of focal adhesions through the mechanical control of Hippo pathway downstream effector Yes Associated Protein (YAP) in breast cancer cells. The stained activation of this co-transcriptional activator has been independently associated to the growth and dissemination of many different tumor types.
Our group also described how YAP aberrant activation following pathological cardiac ECM remodelling plays a pivotal role in heart failure, by contributing to cardiac fibroblast activation and contractility.

In the context of the failing heart, we identified RNA binding proteins which are mechanosensitive and confer mRNA metabolism sensitivity to cardiac ECM mechanical turmoil and impinging on YAP alternative splicing. Finally, by employing bioengineered tools allowing the tight control of cell-matrix interaction and pluripotent stem cells, we also highlighted that mechanosensing is controlled in a stage-specific fashion and contributes critically to phenotype specification.

In conclusion, through a vast array of cellular models and bioengineered tools, we highlighted the pivotal role of mechanical cues in cell function and disease.

keywords: mechanobiology, diseases, heart failure, tumor biology

20941864267

AAV-MEDIATED GENE THERAPY FOR AXON REGENERATION AFTER SPINAL CORD INJURY.

Pavla Jendelova (Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic)

Objectives
The damaged nervous system leads to the impairment of the motor, sensory and autonomic functions, since the central nervous system (CNS) regeneration is very limited. Failure of axon regeneration in the CNS is partly due to the inhibitory environment, and partly due to the intrinsic loss of regenerative ability with neuronal maturation. A key molecule for promoting migration and growth is membrane-associated PIP3, which is produced by PI3Kδ. In mature neurons many axon growth molecules are excluded from axons, and PI3Kδ expression enables anterograde transport of developmentally restricted molecules, such as the integrins, which has been shown to promote growth. The regenerating axons must overcome nonpermissive extracellular matrix of the glial scar, in which, among others, tenascin-C is upregulated after spinal cord injury and contributes to the inhibitory environment around the lesion site.
The migration-inducing tenascin-binding integrin is alpha9beta1, which is expressed in the embryonic nervous system but downregulated in adults and not upregulated after injury.

Methods
In our studies we focus on regeneration of sensory axons and corticospinal tract axons (CST) using two different approaches based on gene therapy. Sensory regeneration was achieved in animals with dorsal column crush lesion using AAV based viral vector delivery of the integrin α9 and kindlin 1 genes to the dorsal root ganglia (DRG). We addressed two different levels of SCI, C4 lesion with DRG C6 and C7 injections for forelimb sensory restoration and T10 lesion with DRG L4 and L5 injections for hindlimb sensory restoration. To regenerate CST, we performed C4 dorsal lesion and injected the right motor cortex at 4 sites concurrently with viral vector mixture of PI3KCD and GFP. Functional outcome was assessed with battery of behavioral tests (tape removal, von Frey, Plantar test, grip strength test, ladder walking and staircase). Regenerating axons were identified by immunostaining.

Results
Significant improvement was observed in Von Frey test for mechanical perception and Hargreaves test for thermal sensation in α9 kindlin 1 treated animals with both, cervical and thoracic lesions when compared to controls. Tape removal test was improved only in treated animals with T10 lesion. Positive behavioural outcome was confirmed by counting axons from α9 and kindlin 1 group above the lesion and c fos staining showing the connectivity of newly grown axons in the treated groups. Significant improvement was detected in paw reaching test and grip strength test in PI3KCD treated animals compared to controls. Expression of PI3Kδ by cortical neurons elicited growth of CST axons at least 1.3 cm below lesion 12 weeks after SCI.

Conclusion
The AAV-mediated gene therapy leads to robust sensory and CST axon regeneration after SCI proved by behavioural tests and immunohistochemical staining.
Supported by: NEURORECON CZ.02.1.01/0.0/0.0/15_003/0000419

keywords: gene therapy, axon regeneration, extracellular matrix, sensory nerves, corticospinal tract

83767252566

GENERATION AND CHARACTERIZATION OF HUMAN IPSC-DERIVED CARDIAC ORGANOIDS FOR TRANSLATIONAL MEDICINE

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Cardiovascular diseases (CVD) remain as the leading cause of death worldwide, and there is an increasing focus on developing physiologically relevant in vitro cardiovascular tissue models suitable for studying personalized medicine and pre-clinical tests. While recent technologies provide some insight into how human CVDs can be modelled in vitro, they may not always give a comprehensive overview of the complexity of the human heart due to their limits in cellular heterogeneity and physiological complexity[1][2][3]. Furthermore, animal models may not always faithfully reflect the features that are unique to human biology and disease [4].

We have optimized a scaffold-free protocol to generate multicellular, beating, self-organized and functional human cardiac organoids (hCOs) derived in vitro from induced pluripotent stem cells (iPSCs). The hCOs contain multiple cell types of the heart, are viable for more than 50 days, and show functional beating response without external stimuli. We have shown that hCOs have improved cardiac specification, survival and maturation compared to standard 2D monolayer cardiac differentiation. Furthermore, the hCOs has shown functional beating response to cardioactive drugs, and could be used to model chemotherapy-induced cardiotoxicity. Our study demonstrates that culture dimensionality and time are important for survival, differentiation, and maturation of in vitro cardiac tissue models in long term, and could enable further possibilities in translational research in cardiovascular biology.

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keywords: cardiovascular biology, 3D-cell culture, stem cells, organoids, microtissues, tissue engineering, cardiotoxicity

52354553526

ELECTROSPUN SILICA NANOFIBRES AS MULTIFUNCTIONAL SUBSTRATE FOR DRUG DELIVERY AND TISSUE REGENERATION

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Introduction
Over the last two decades, electrospun nanofibres were demonstrated to be interesting material applicable in regenerative medicine and drug delivery, possessing number of unique properties including high specific surface area, high porosity and small pore size. Properties such as chemical and mechanical stability, biocompatibility and degradation kinetics then depend on chemical composition, crosslinking or possibly functionalization. While polymer nanofibres may exhibit serious disadvantages including swelling in moist environment and during degradation or low surface functionality or limited bioactivity, inorganic nanofibres represent a family of nanofibres unlimited by these factors and potent for medical applications. Silica nanofibres, being member of this family, combine traditional properties of nanofibres based on their structure and advantages of inorganic bioactive material. The aim of this paper is to outline properties and performance of silica nanofibres as biocompatible, biodegradable, and easy to modify high performance material for regenerative medicine and drug delivery.

Methodology
Silica nanofibres were prepared by sol-gel method and needle-less electrospinning, which led to formation of nanofibrous matrix of 5 – 30 g/m2 and mean fibre diameter 180 – 850 nm depending on the spinning conditions. Biocompatibility was tested in vitro on several model cell lines including 3T3-A31 fibroblasts, Hacat keratinocytes, Vero cells and HepG2 hepatocyte-like cells in compliance to ISO 10993-5. Biodegradation of silica nanofibres was evaluated in vitro under simulated conditions (37 °C, SBF). Silica release into the SBF was measured using ICP-MS. Impact of degradation on the surface morphology was evaluated by electron microscopy (SEM). Surface availability for functionalization and its impact on relevant properties was tested by APTES aminosilane silanization.

Results
Silica nanofibres, obtained by electrospinning, were confirmed to promote multitissue biocompatibility in vitro. Fast degradation under simulated conditions in vitro was observed with surface erosion appearing after 24 hours in simulated body fluid (SBF) with limited swelling and sustained integrity of the nanofibrous matrix. Silica released upon degradation in form of orthosilicic acid, was confirmed to have a beneficial impact on cellular proliferation in vitro, which is known effect provided silica nanomaterials in general. Successful grafting of –NH2 amino group by silanization of surface silanol group was confirmed without relevant impact on the fibre morphology or integrity. Positive impact of the silanization process on biocompatibility was verified.

Conclusion

The electrospun silica nanofibres were confirmed as biocompatible and bioactive nanomaterial with capacity to promote tissues regeneration through its degradation products. Moreover, the unique degradation mechanism reminiscent of Stöber silica degradation mechanism was revealed. The high specific surface available for surface functionalization, can be applied in drug and bioactive molecules delivery in wound healing and tissue regeneration.

keywords: silica, nanofibres, electrospinning, biodegradation

52354540244

PRODUCTION OF UNIFORM ORGANOIDS IN MICROFLUIDIC CHIPS AND AND THE INTERACTION WITH CAPILLARIES

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Organoids, 3D multicellular aggregates, provides excellent possibilities to recapitulate pato/physiological properties of human tissues or organs. Methodological obstacles lie in uniform formation, differentiation and long-term cultivation. Preparation and maintenance of organoids can be labor-intensive, medium exchange is usually discontinuous, and individual organoids are highly heterogenous in size, morphology, and cellular composition. Microfluidics help to overcome some of these disadvantages. Due to automation and proper design of chips, microfluidic systems are capable of continual medium flow in space and time, which allows to create better controlled microenvironments for cells.
In this work, we present designs of microfluidic system for uniform formation of organoids with low divergence of size and shape. System enables a parallel perfusion culture of large amount of cell spheroids as well as long term cultivation and cell differentiation. Upon in silico simulations, we optimized conditions for long-term cultures in chip. To improve their nutrient support, we also examined approaches for their vascularization.

This work was supported by the European Regional Development Fund - project INBIO (No. CZ.02.1.01/0.0/0.0/16_026/0008451), project from Masaryk University (MUNI/IGA/1297/2021), Czech Science Foundation GA21-06524S and Ministry of Health of the Czech Republic (NU21-08-00561).

keywords: organoids, vascularization, microfluidics, 3D bioprinting

S61
Tissue Engineering in Microgravity for Health in Space and on Earth
Room: S4 B
(1 Jul 2022, 11:00 - 12:30)

Conveners: Jeremy Teo, Cian O' Connor

41883620517

TISSUE DENSITY DIMINISHES THE EFFECTS OF SIMULATED MICROGRAVITY ON DENDRITIC CELL IMMUNE POTENCY IN VITRO

Mei Elgindi (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Anna Garcia-Sabaté (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Jiranuwat Sapudom (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Mohamed Al-Sayegh (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Weiqiang Chen (New York University, New York, United States), Jeremy Teo (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates)

Interest in manned spaceflights has increased in recent years and this brings about a surge in the need for more in-depth research into the adverse effects of spaceflights on the human body. In the absence of gravitational forces there are countless negative effects on the immune system. This dysfunction in the immune system can lead to increased susceptibility of infections by astronauts and poor wound healing. In particular, there is a lack of research on the effects of microgravity on immune cells in physiologically relevant cellular microenvironments. Amongst the various immune cell types, dendritic cells (DCs) are the primary mediators between the innate and adaptive immune systems and any dysregulation of these cells can lead to inadequate immune responses, especially in a long-term specific immune response. To gain insight into how DCs' function is affected under microgravity conditions on Earth, we utilized loose and dense 3D fibrillar collagen matrices to explore the effects of simulated microgravity using Random Positioning Machine (RPM) on cells of the immune system. Immune potency of DCs was assessed in terms of their transcriptome profile, differentiation state, secreted cytokine profile, antigen uptake, and their ability to trigger a T-lymphocyte cell response. The transcriptome profile, using RNA-sequencing, showed that DC differentiation and maturation were altered under simulated microgravity conditions in a matrix density dependent manner. In addition, surface markers, cytokine secretion profile, and functional assays of DCs were reduced upon exposure to simulated microgravity conditions. Overall, our work pinpoints the importance of mechanotransduction in DC differentiation and function under simulated microgravity conditions, which could contribute to the design of immune modulating materials for use in spaceflight. In addition, as microgravity-associated physiological alterations closely resemble those found in the elderly, this data is not only important for space biology but could also be beneficial for a simulated model of aging on Earth.

keywords: Space Biology, Mechanobiology, Dendritic cells, 3D collagen

62825419928

3D MICROENVIRONMENT MAINTAINS THE TRANSCRIPTOME PROFILE OF T CELLS BUT NOT B CELLS IN SIMULATED MICROGRAVITY

Mei ElGindi (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates) , Jiranuwat Sapudom (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Jeremy Teo (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Anna Garcia-Sabate (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Marc Arnoux (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Nizar Drou (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates)

The adverse effects of space travel on the human body are abundant and with the rise in interest in manned spaceflight over recent years, there is an increased need for more research into these effects. In the absence of gravitational forces, one of the primary systems to be negatively affected is the immune system and its dysregulation leads to increased susceptibility of astronauts to infections. Lymphocytes (T and B cells), the key players of the adaptive immune system, are involved in fighting infections and producing antibodies. Currently, less is known to which extent microgravity affects lymphocyte functions. To address the above mentioned question, we utilized 2D tissue culture plastic and 3D collagen matrices, the latter better mimics the in vivo cellular microenvironment, as cell culture models. T and B cells were cultured on ground and under simulated microgravity conditions. Both cell types were also analyzed under resting and activated states using RNA-Sequencing. Our data indicates that the 3D culture microenvironment appears to maintain the transcriptome profile of T cells but not B cells during early activation under simulated microgravity conditions when compared to ground controls. In T cells, DNA damage and protein degradation were upregulated in 2D cell culture under simulated microgravity conditions whereas the 3D microenvironment prevents these adverse effects. Interestingly, B cells showed a higher number of differentially expressed genes when activated in 3D collagen matrices compared to 2D cell culture. However, simulated microgravity conditions attenuates these effects. Overall, our results suggest that the cellular microenvironment plays a role on lymphocyte behavior on Earth and in simulated microgravity.

keywords: microgravity, lymphocytes, transcriptome, immunology

20941860328

STUDIES OF CELLULAR DIFFERENTIATION IN SIMULATED MICROGRAVITY REVEAL AN IMPORTANT ROLE FOR Β-ACTIN IN MECHANOSENSING

Oscar Sapkota (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Tomas Venit (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates), Piergiorgio Percipalle (New York University Abu Dhabi, Abu Dhabi, United Arab Emirates)

Introduction
Mechanotransduction is mediated by the actin cytoskeleton and it is important in helping stem cells determine their fate. Differentiating cells assess changes in mechanical stressors in the extracellular matrix (ECM) and transduce them into a reaction cascade to regulate genes involved in cellular lineage specification. During differentiation, the nuclear β-actin pool plays an important role in regulating gene expression as part of the chromatin remodeling complex BAF and bound to all three eukaryotic RNA polymerases. Based on these considerations, the aim of this study is to investigate whether the nuclear β-actin pool is involved in regulating gene expression that controls mechanosensory pathways in response to mechanical unloading in the ECM.

Methodology and results
Wild-type (WT) and β-actin knock-out (KO) mouse embryonic fibroblasts (MEFs) were subjected to simulated microgravity (sμG) for 72 hours using a Random Positioning Machine (RPM) to study changes in their global epigenetic landscape. This was achieved by staining MEFs with antibodies against active, repressive, and enhancer-associated histone marks and analyzing their intensity and distribution inside cell nuclei using the Cellomics CX7 Laser High Content Phenotypic Platform. Results show an increase in repressive chromatin marks (H3K9Me3, Hp1a) and dysregulated levels of enhancer-specific epigenetic marks (H3K27Ac, H3K4Me) in the β-actin KO but not in the WT cultured in sμG. This confirms a role of β-actin in regulating heterochromatin and hints to a novel role for the nuclear beta actin pool in the regulation of enhancers to transduce microgravity-induced changes. To examine this further, total RNA was extracted from WT and β-actin KO MEFs cultured in sμG for 24 and 72 hours and subjected to RNAseq. Analysis of the transcriptomic data obtained shows upregulation of genes involved in focal adhesion, osteogenesis, and axon guidance in the WT under sμG, but not in the KO.

Conclusion
Considering the observed epigenetic and transcriptomic changes observed upon β-actin depletion, we propose that β-actin regulates expression of genes involved in mechanosensing. We speculate that β-actin performs this novel function by controlling heterochromatin levels and potentially impacting on enhancers' activity.

keywords: Mechanotransduction, microgravity, β-actin, gene regulation

S62
Tissue regeneration by integration of bioinspired materials
Room: S4 B
(29 Jun 2022, 11:00 - 12:30)

Conveners: Sandra Van Vlierberghe;
Heungsoo Shin

31412741608

THE CONTROLLED DELIVERY OF PROTEOGLYCAN-4 IN A SCAFFOLD-BASED SYSTEM FOR CARTILAGE REPAIR APPLICATIONS

Austyn Matheson (Royal College of Surgeons in Ireland, Dublin, Ireland), Eamon Sheehy (Royal College of Surgeons in Ireland, Dublin, Ireland), Tom Hodginkson (Royal College of Surgeons in Ireland, Dublin, Ireland), W. Michael Scott (University of Calgary, Calgary, Canada), Tannin Schmidt (University of Connecticut, Health Campus, Farmington, United States), Fergal O'Brien (Royal College of Surgeons in Ireland, Dublin, Ireland)

Introduction
Proteoglycan-4 (PRG4) is a mucinous glycoprotein with critical roles in the bio-lubrication of articular cartilage and joint health1. Intra-articular injection of PRG4 protects against cartilage deterioration in in vivo models of osteoarthritis, an effect that is largely attributed to restored bio-lubrication. Thus far, tissue-engineered, collagen-glycosaminoglycan (coll-GAG) scaffolds2 developed by our lab have shown significant promise in treating focal yet critical-sized osteochondral defects within several in vivo animal models and in humans Such scaffolds alone, however, lack the requisite frictional qualities of articular cartilage. The regenerative capacity of collagen-based biomaterials can be improved through the incorporation of therapeutic molecules4 and we have previously demonstrated that the frictional properties of coll-GAG scaffolds are enhanced by soaking scaffolds in recombinant human (rh)PRG4-solution in vitro3. The goal of this study was, therefore, to investigate an alternative approach to PRG4-injections by developing a scaffold-based recombinant human (rh)PRG4 delivery system for superior bio-lubrication of cartilage biomaterials.

Methods
1) The effect of rhPRG4 (Lμbris Biopharma3) treatment on mesenchymal stem cell (MSC) proliferation (DNA, Picogreen) and sulfated-GAG (sGAG, Blyscan Assay) production was examined in 2D-culture (wells). 2) Subsequently, rhPRG4 was incorporated via bulk-addition to a collagen I-hyaluronan slurry (blended mixture) and lyophilized, previously optimized for controlled molecule release4. The coll-rhPRG4 scaffold properties were assessed including pore architecture (JB4, toluidine-stain), rhPRG4 release (ELISA) and molecular weight (western blot). 3) The scaffolds' mechanical properties including the bio-lubrication (coefficient of friction3, COF) and compressive stiffness were characterized. 4) Finally, the biological response of MSCs in 3D-culture (scaffolds) after 14, 21, and 28 days was assessed.

Results
1) In 2D, rhPRG4-treatment significantly increased MSC proliferation (+61%) and sGAG production (+4.3x). 2) rhPRG4 was successfully incorporated within scaffolds as demonstrated by western blots indicating intact rhPRG4 of molecular weight ~460kDa, rhPRG4 released gradually from the scaffold up to day 14 (67% release by day 7). The novel biomaterial was highly porous with uniformly interconnected pores (121.9 to 160μm). 3) The coll-rhPRG4 scaffolds were lubricious, with a 42% reduction of COF (Coll-GAG-rhPRG4: 0.068 ±0.01 vs. Coll-GAG: 0.118±0.02, $p<0.001$), however incorporation of PRG4 reduced the stiffness of the scaffolds. 4) Coll-GAG-rhPRG4 scaffolds produced 40 to 80% more sGAG than controls across multiple timepoints (Days 14, 21, and 28).

Conclusion
An innovative scaffold based rhPRG4 release system was successfully designed for cartilage repair applications. Additionally, beyond rhPRG4's ability to support bio-lubrication, a previously undescribed biological effect was revealed, rhPRG4 increased cell proliferation and sGAG formation in vitro. Therefore, PRG4 containing biomaterials may be suitable for other applications (e.g., contact lens materials). To conclude, scaffold-based delivery of PRG4 may circumvent the need for multiple intra-articular injections whilst also providing cues to promote cartilage regeneration.

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Acknowledgements
ReCaP: European Research Council Advanced Grant (Grant: 788753), National Science Foundation- Science Foundation Ireland (NSF-SFI) (Grant: NSF_ 17_US_3437), ADMIRE Marie Sklodowska-Curie-Action-Cofund (EU Horizon 2020: 945168, SFI: 12/RC/2278_2)

keywords: PRG4, Scaffolds, Biolubrication, Lubricin, Cartilage

73296310004

HYBRID 3D-PRINTED HYDROGEL SCAFFOLDS FOR LIVER TISSUE ENGINEERING

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Introduction
Annually, millions of people die because of liver failure, while the waiting duration for a donor liver is around 12 months.[1] Herein, we target hybrid 3D-printed scaffolds to serve liver tissue engineering(LTE) applications.
As starting materials gelatin in combination with a polysaccharide was used to develop printable hydrogels. As polysaccharides dextran (Dex) and chondroitin sulphate (CS) were selected as mimics for the liver extracellular matrix (ECM) to explore their effect on the cell response. Methacrylated gelatin (GelMA) served as benchmark. The hydrogel materials were characterized on 2D-as well as on 3D-level.
HepG2 cells were used to assess the in vitro biocompatibility of the developed hydrogels.

Materials and methods
Thiolated gelatin (GelSH)[2] was crosslinked with the norbornene-functionalized polysaccharides DexNB and CSNB. Gelatin was methacrylated using methacrylic anhydride (GelMA) as reference material.
The different materials were characterized on both 2D- and 3D-level to assess the physico-chemical properties and the biocompatility. 3D-hydrogel scaffolds of the materials were developed by indirect 3D-printing[3].

Results and discussion
On a 2D-level, DexNB-GelSH and CSNB-GelSH were superior over GelMA as their crosslinking kinetics were significantly faster and they mimicked natural liver tissue (NLT) to a greater extent with respect to swelling and mechanical properties. The swelling ratio of GelMA, DexNB-GelSH and CSNB-GelSH were respectively 9.1±0.5 and 9.6±0.5 and 8.7±0.2 which is in line with the swelling of NLT (i.e.10)[3].
Atomic Force Microscopy (AFM) measurements revealed superior microscale mechanical properties of the DexNB-GelSH hydrogel sheets compared to the other materials. DexNB-GelSH exhibited a stiffness of (196±24)kPa, CSNB-GelSH of (106±2)kPa and GelMA of (291±11)kPa. Healthy NLT exhibits an average surface stiffness of (183±48)kPa. The higher the stiffness, the more the material mimics the ECM of unhealthy cirrhotic liver ((411± 63)kPa)[4].
On a 3D-level, DexNB-GelSH scaffolds exhibited a compressive modulus of (4.8±1.6)kPa which is in excellent agreement with that of NLT (i.e. 1–5kPa)[5] as compared to GelMA which resulted in a modulus of (8.5±1.9)kPa and CSNB-GelSH (12.6±1.9)kPa.
So far, the biocompatibility of CSNB-GelSH and GelMA were compared. However, the in vitro biocompatibility of both materials was comparable based on the MTS assay. The live-dead staining showed that the cells grew more into clusters on the DexNB-GelSH scaffolds compared to the more spread morphology which the cells exhibited on the GelMA material.

Conclusions and future perspectives
DexNB-GelSH and CSNB-GelSH scaffolds are promising hybrid materials to support LTE as they exhibit similar physico-chemical properties compared to NLT (cfr. microscale stiffness, compressive modulus, swelling ratio and chemical compostion), while cell viability and proliferation of the hepatocytes were preserved.
In future research, different cell types such as primary hepatocytes and organoids will be included in the biological evaluation. Furthermore decellularized liver ECM will be incorporated into the hydrogel material in order to improve the cell interactivity and proliferation.

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keywords: 3D-scaffolds, Liver tissue engineering, Hybrid hydrogels, hepatocytes

94238101804

COMBINING PROTEOLYTIC SEQUENCES, VEGF-MIMETIC PEPTIDE AND LAMININ-DERIVED PEPTIDE WITHIN ELASTIN-LIKE RECOMBINAMER SCAFFOLDS FOR THE SPATIOTEMPORAL DIRECTION OF ANGIOGENESIS AND NEUROGENESIS

Fernando González-Pérez (Universidad de Valladolid, VALLADOLID, Spain), Matilde Alonso (Universidad de Valladolid, VALLADOLID, Spain), José Carlos Rodríguez-Cabello (Universidad de Valladolid, VALLADOLID, Spain)

Introduction
The fabrication of three-dimensional (3D) scaffolds able to promote a spatiotemporal guidance of cell infiltration, vascularization and innervation are of great interest in tissue engineering and regenerative medicine (TERM) applications. To this end, peptide sequences displaying fast and slow proteolytic rates towards urokinase plasminogen activator uPA, namely GTAR and DRIR, the QK vascular endothelial growth factor mimetic peptide and the IKVAV laminin-devired peptide for neuronal adhesion and proliferation have been described in literature as promising candidates [1,2]. Hydrogels based on Elastin-like recombinamers (ELRs) also have shown applicability in this regard, as their tailorable recombinant nature allow the genetic encoding of proteolytic sequences into their backbone and their peptide sequence enable the covalent tethering of bioactive residues such as the QK peptide or IKVAV.

Methodology
The ELRs prepared in this work were decorated with the GTAR or DRIR proteolytic sequences, the RGD cell-adhesion domain, the QK pro-angiogenic peptide or the IKVAV pro-innervation peptide. Furthermore, click catalyst-free crosslinkable domains were attached to the ELRs to produce the intended hydrogels. In vitro studies allow to determine the effect of IKVAV peptide over C6 glial cells adhesion, whereas the porous structure of the prepared hydrogels was evaluated by microscopic techniques. To assess the ability of ELRs to promote angiogenesis and neurogenesis, we fabricated a 3D construct containing two different cylindrical ELR hydrogels. In detail, the first cylinder contains the QK peptide with the GTAR fast-proteolytic sequence, whereas the second cylinder contains the IKVAV peptide with the GTAR fast-proteolytic sequence, in order to evaluate both bioactivities. In contrast, the outer part lack or display the DRIR slow-proteolytic sequence. Cell infiltration, vascularization and innervation were analyzed by histology and immunohistochemistry (IHC) upon subcutaneous implantation in Swiss CDR-1 mice with time.

Results
Microscope analysis showed the porous structure of the fabricated hydrogels. In vitro studies confirmed the effect of the IKVAV peptide over the cell-adhesion of C6 glial cells. Furthermore, in vivo studies of 3D ELR models revealed a marked increase in cell colonization in the interior tubes containing fast-proteolytic sequences, when compared to the outer part lacking or bearing slow-proteolytic sequences. Histology and IHC results showed the effect of the QK peptide triggering angiogenesis, and the effect of the IKVAV peptide triggering innervation in the pre-design orientation

Conclusions
The combination of proteolytic-sensitive sequences, the QK pro-angiogenic peptide and the IKVAV pro-innervation peptide into 3D ELR hydrogels confirmed the ability to spatiotemporally control angiogenesis and innervation in vivo. Specifically, the cylinder displaying the QK peptide

promote a faster endothelialization, whereas the cylinder displaying the IKVAV peptide promote a faster innervation, following the pre-designed orientation. These results set the basis for the development of ELR-based scaffolds for TERM applications where the spatiotemporal control of vascularization and innervation play an important role.

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keywords: Elastin-like recombinamer, hydrogel, innervation, vascularization, proteolytic sequences

41883630105

TIME COURSE OF ECTOPIC BONE FORMATION IN RATS INDUCED BY RHBMP6 WITHIN AUTOLOGOUS BLOOD COAGULUM WITH CALCIUM PHOSPHATE CERAMIC PARTICLES

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Introduction
Osteoinductive bone morphogenetic proteins (BMPs) possess the ability to induce bone formation and therefore have been the basis of osteoinductive devices designed for bone regeneration. Osteogrow C is a novel autologous bone graft substitute comprised of recombinant human Bone Morphogenetic Protein 6 (rhBMP6) within autologous blood coagulum (ABC) with synthetic calcium phosphate (CaP) ceramic particles. CaP particles serve as a compression resistant matrix and are available in a broad range of shapes and sizes. The aim of this study was to investigate the time course of ectopic bone formation in rats following subcutaneous implantation of rhBMP6/ABC with CaP particles in a size range from 2360 to 4000 µm.

Methodology
Osteogrow C osteoinductive device was prepared as follow: rhBMP6 (20 µg per implant) was added to autologous blood (500 µL), mixed with synthetic ceramic particles (size range: 2360-4000 µm; chemical composition: TCP/HA 80%/20%; porosity: 86%; average pore size: 246 µm) and left to coagulate at room temperature. Subcutaneous pockets were created in the axillary region of Sprague Dawley rats (male, 6-8 weeks, 250-300 g), and following blood coagulation, osteoinductive devices were implanted in pockets. Animals were killed on days 7, 14, 21, and 35 following implantation. Extracted implants were analyzed on histological and microCT sections to investigate the time course of ectopic bone formation.
Results: MicroCT analyses revealed that Osteogrow C implants induced extensive bone formation two weeks after implantation at rat ectopic site. Histological analyses have shown that seven days after implantation large areas of endochondral ossification were present only at the peripheral parts of the implants while on day 14 endochondrally formed bone was present throughout the implant between ceramic particles. On day 21 following implantation BMP-induced osteogenesis has reached its final stage and ectopic bone was present at the ceramic surfaces, in the pores, and between the particles. At the end of the observation period (day 35) the structural properties of newly formed bone were similar as on day 21, however, the thickness of trabeculae between the particles was decreased while the number of adipocytes was increased and they became the predominant cell population in the bone marrow.

Conclusions
In the present study we have elucidated dynamics of ectopic bone formation following implantation of rhBMP6 in ABC with ceramics (Osteogrow C). Osteogrow C implants with large (2360-4000 µm) ceramic particles induced bone in rat ectopic site proving excellent osteoinductive properties of tested implants. Therefore, Osteogrow C is a promising novel therapeutic solution for bone regeneration.

keywords: Bone morphogenetic proteins, Bone regeneration, Ceramics, Tricalcium phosphate, Hydroxyapatite

73296363497

PROGNOSTIC EVALUATION OF THE USE OF THREE-DIMENSIONAL (3D) SCAFFOLDS ON CHRONIC SKIN LESIONS USING NEW BIOMEDICAL IMAGING TECHNOLOGIES.

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Introduction
Human umbilical cord blood stored in blood banks cannot always be used for hematopoietic stem cell transplantation as up to 80% of stem cells are lower than the cut-off required for such application, being potentially available for non-transfusion applications such as the source of platelet growth factor [1]. In this study, we fabricated a fibrin-based drug delivery system to provide a local and sustained release of cord blood platelet lysate (CBPL) at the wound site. Also, we assessed the temporal evolution of skin lesions using photoacoustic imaging (PAI) and near-infrared spectroscopy (NIRS), non-invasive technologies translatable to a clinical setting. [2,3]

Methods
Fibrin scaffold loaded with CBPL was fabricated by a peculiar spray process (IT Patent application pending N. 102021000025664). Excision wounds were created on the dorsum of genetic diabetic mice (db/db) using a biopsy punch (8 mm diameter). Wounds were treated with fibrin scaffolds with or without CBPL. Oxygen saturation (%SO2) was monitored in the wound area and surrounding tissue through PAI and NIRS imaging on days 0, 7, and 14 after lesion induction. At each time point, whole blood was collected for flow cytometry, and on day 14 wound tissue was retrieved for histological analyses.

Results
Diabetic mice treated with CBPL scaffold showed a significantly higher closure of about 82% of the initial lesion size, 14 days after the intervention. Animals treated with the unloaded scaffolds showed closure of only 62% of the initial lesion size. Histological analysis demonstrated an improved reepithelization and collagen deposition in granulation tissue in mice treated with CBLP scaffold in comparison to unloaded fibrin scaffold. The flow cytometric quantification of circulating fraction variations (compared to baseline) of endothelial progenitor cells (EPCs, CD45-/CD34+/KDR+) showed an increase in the CBPL fibrin scaffold treated mice when compared with the fibrin scaffold alone. Moreover, a statistically significant correlation ($P = 0.03$, $R = 0.754$) has been observed on day 14 after induction of skin lesion between wound repair areas and the variations of EPCs fraction in the CBPL fibrin scaffold treated mice. In all the lesions, the %SO2 signal from both PAI and NIRS showed a typical trend characterized by an increase one week after the wound induction (day 7) followed by a decrease toward the initial intact skin %SO2 values (day14).

Conclusions
This study allows developing a new approach based on imaging and circulating biomarkers to describe the inflammatory state and healing dynamics during the regenerative process. The

present findings pave the way for clinical translation of the developed experimental patches and combined molecular imaging.

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keywords: wound healing, growth factor, fibrin, photoacoustic imaging, near infrared spectroscopy

62825448726

HEPARAN SULPHATE ANALOGUE HYDROGELS AS A PLATFORM FOR KIDNEY ORGANOID MATURATION

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Kidney organoid derived from pluripotent stem cells (PSCs) have gradually become a platform to understand kidney morphogenesis, development and diseased states. Despite the abundant differentiation protocols to obtain relevant renal cell types and organoids, most of these only reach a developmental immature stage (1). We hypothesize that the limited maturation is due to absence of relevant ECM molecules that interact with signalling of the developing kidney. With this, we synthesized heparan sulphate analogue hydrogels to observe if organoid maturation will proceed upon encapsulation. Precursor molecules were successfully functionalised with both 5.2% norbornene, and 1.7 SO3 groups per disaccharide of backbone. Adsorption studies on these hydrogels show strong affinity towards growth factors relevant to the reciprocal induction signalling in the developing kidney. This demonstrates the materials' mimicry on ECM modulated signalling of heparan sulphate. In addition, further maturation of encapsulated organoids was observed by means of immunocytochemical staining with the presence of principal and intercalated cell populations, along with reduced interstitial cells. Overall, organoid maturation may be realised with these class of materials with a more physiological model for drug discovery, disease modelling, and understanding further the developmental biology of kidney.

Reference

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keywords: Hydrogels, In vitro model, organoids, kidney

41883603688

DIRECTING STEM CELL COMMITMENT IN 3D BIOINSPIRED HYDROGELS BY GROWTH FACTOR SEQUESTRATION USING MOLECULARLY IMPRINTED NANOPARTICLES

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Introduction
Growth factors (GFs) are a key component of tissue engineering, but their exogenous administration has proven costly and ineffective. Extracellular matrix-inspired biomaterial approaches have sought to sequester these molecules, regulating their activity and presentation to cell receptors.1 Our previous work has shown that molecularly imprinted nanoparticles (MINPs) can play this role in standard 2D and 3D cell cultures, combining high recognition specificity, stability, and cost-effectiveness.2 Taking this concept a step forward, here we tested MINPs against transforming growth factor (TGF)-β3, a regulator of tenogenesis, in hydrogel systems with bioinspired ordered microstructures. Our hypothesis is that combined control over biophysical and biochemical cues will synergistically contribute to more robust tenogenic commitment of stem cells.

Methodology
A TGF-β3 N-terminal epitope was used as template molecule for solid phase imprinting by polymerization of acryloyl-containing monomers. MINP affinity and selectivity were assessed by surface plasmon resonance (SPR), Western blot and dot blot. Aligned polycaprolactone meshes were first produced by electrospinning, followed by cryosectioning into 50-μm microfibers. To enable their remote orientation within hydrogels, superparamagnetic iron oxide nanoparticles were synthesized by thermal decomposition and incorporated in the electrospinning solution. Finally, tenogenic constructs were prepared by encapsulating human adipose tissue-derived stem cells (hASCs), along with microfibers and MINPs, in transglutaminase-crosslinked gelatin

hydrogels. Microfibers were unidirectionally aligned by applying a uniform magnetic field during gelation.

Results
SPR demonstrated a remarkable affinity of MINPs for the template (KD = 18±13 nM), in the range of some monoclonal antibodies. In comparison, the interaction between TGF-β3 epitope and MINPs imprinted against biotin was negligible, demonstrating the impact of imprinting on the molecular recognition potential of nanoparticles. hASCs remained viable for at least 14 days in hydrogel systems, showing a preferential orientation along the microfiber alignment axis. Preliminary qPCR findings indicate a positive correlation between MINP concentration and tendon-associated gene expression markers (SCX, TNMD) in aligned constructs, which is not observed in gels with randomly oriented microfibers. Furthermore, osteogenesis-associated ALP expression was downregulated as MINP concentration increased, corroborating the hypothesis of phenotypic steering toward tenogenesis. Protein synthesis is currently being analyzed by immunoassays to further bolster these results.

Conclusions
Our findings demonstrate the potential of molecular imprinting as a cost-effective complementary strategy in tissue engineering approaches. The endogenous GF sequestering ability of MINPs allows an efficient replacement of expensive recombinant GFs. Moreover, we also show that its combination with microstructural cues synergistically drives stem cell differentiation toward tenogenesis in engineered constructs. Thus, this strategy not only holds potential to significantly improve tendon healing after injury, but its principles can also be applied to engineer different tissues.

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Authors declare no conflicts of interest.

keywords: growth factors, hydrogels, microstructure, molecular imprinting, stem cells

S63
Towards automated technologies for organoid-based tissue biomanufacturing
Room: S3 B
(30 Jun 2022, 13:30 - 15:00)

Conveners: Ioannis Papantoniou

20967801764

PREDICTIVE ANALYSIS OF CARDIAC MICROTISSUE MANUFACTURING BY MONITORING METABOLIC CQAS

Sean Palecek (University of Wisconsin - Madison, United States)

Biomanufacturing cells and tissues from human pluripotent stem cells (hPSCs) typically strives to guide differentiation through developmentally relevant pathways in a well-defined, dynamic bioreactor environment. While great strides have been made in differentiating hPSCs to many somatic cell types, robust biomanufacturing remains a roadblock to clinical progress of hPSC-derived cell and tissue therapies. In particular, scaling manufacturing to meet clinical needs, reducing cost, improving cell phenotypes, and improving process robustness are critical challenges. hPSC-derived cardiomyocytes have tremendous potential to restore cardiac function to heart failure patients. However, these cells suffer from poor survival and functional integration in preclinical models of heart disease. We have developed protocols to differentiate hPSCs to endothelial cells and cardiac fibroblasts, and demonstrated that inclusion of these cells during cardiomyocyte biomanufacturing accelerates acquisition of maturation phenotypes such as morphology, sarcomere protein expression, and calcium handling in the cardiomyocytes. Importantly, these heterotypic cell interactions must be provided to cardiac progenitor cells, allowing the cell types to co-differentiate. To reduce costs and improve scale of cardiomyocyte biomanufacturing, we have transitioned 2D cardiomyocyte differentiation to 3D, reducing cost by approximately 85% and permitting manufacturing of greater than one trillion cardiomyocytes in a 300 mL spinner flask bioreactor. To improve biomanufacturing process robustness, we have performed a multi-omic characterization of differentiating cardiomyocytes and utilized unbiased data analytics to identify genes, proteins, and metabolites that when measured before day 5 predict successful vs. failed batches at day 15, determined by the percentage of cells expressing cardiac troponin T. We envision that these multivariate predictive critical quality attributes can be used to more quickly identify failed batches and eventually lead to closed-loop control strategies to improve biomanufacturing process robustness.

keywords: human pluripotent stem cells, cardiac, biomanufacturing, metabolic

62825429844

AUTOMATED MANUFACTURING OF MICROTISSUE BASED OSTEOCHONDRAL IMPLANTS: THE »JOINTPROMISE« PLATFORM

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Over 300 million cases of osteoarthritis were reported in 2017, stating one of the most prevalent chronic joint diseases worldwide characterized predominantly by long-term progressive cartilage and subchondral bone degeneration. Conventional therapy approaches utilize pharmacotherapy mostly for pain relief and at end stage disease treated by whole joint replacement surgery to retrieve some mobility and function. Novel Regenerative Medicine (RM) strategies employing Tissue Engineered implants could enable cure, more than care, of such life-constraining disabilities to meet the rising demand for medical interventions due to an ageing world population. Engineering joint tissue implants for the regeneration of the cartilage-bone unit of the joints remains a challenge due to the complex structural organization and functionality of native joint tissue. The use of microtissue/spheroid platforms has enabled differentiation and maturation of cartilage intermediates and gives hope for the engineering of efficient large-scale implants for osteochondral regeneration. However, there is still lack of enabling technologies for scaling of these approaches and robust manufacturing with end-to-end automation of such advanced therapeutic medicinal products (ATMPs).

To allow sufficient scaling, overcome risks of contamination as well as inconsistent product quality in manual production procedures, the automated, GMP-compliant manufacturing platform »JointPromise« is developed. By establishing a robust, large-scale manufacturing process, a reliable microtissue-based product for the treatment of deep osteochondral defects can be generated with suitable productivity. The platform concept is based on the translation of Standard Operating Procedures (SOPs) for microtissue production, harvest and condensation into a sequence of automated process steps. Derived process design criteria and technical specifications result in device requirements for an automated production process. After initiating the conceptualizing stage of the platform design by creating a 2D layout according to the material flow of the translated SOPs, the final arrangement of devices was optimized in the overall 3D CAD model. The resulting production platform model combines all required devices for cell cultivation, microtissue harvest and ATMP production in an overall layout consisting of pipetting units, an incubator, centrifuge, bioprinter and housing for a defined hygienic environment. Following the SOPs, about 28,000 microtissue spheroids can be produced within 21 days of culture out of 1 mL cell suspension per tissue culture plate. To reach the required productivity of around 100 tissue culture plates per implant, the production platform will need to process around 70 L of liquids during seeding and harvest processes and 5 L per cell media change to produce around 2.8M microtissue spheroids in 21 days. The build-up of the »JointPromise« platform is followed by the implementation of the control software COPE (Control Operate Plan Execute, Fraunhofer IPT, Aachen, Germany) for process controlling and monitoring during cell seeding, cultivation and harvest.
This project received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement no 874837.

keywords: Automation, Osteoarthritis, Tissue Engineering, Stem Cells, Regenerative Medicine

62825467239

CARTILAGINOUS MICROTISSUES MERGED WITH TAILORED MELT ELECTROWRITTEN MESHES RESULT IN BONE FORMING BIOHYBRIDS

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Introduction
Progenitor cells from the periosteum are major contributors in fracture healing with contribution to the formation of the cartilaginous fracture callus. It was previously demonstrated that microspheroids of human periosteum derived cells, differentiated towards the chondrogenic lineage, could be assembled into scaffold-free constructs that healed murine critical-size long bone defects [1]. However, the stability of such scaffold-free implants can be compromised when scaling-up. In this work, cartilaginous microspheroids were combined with tailored melt electrowritten (MEW) meshes to create an engineered biohybrid callus-like membrane able to induce bone formation via endochondral ossification.

Methods
Human periosteum-derived cells (hPDCs) were seeded in non-adherent microwells (AggreWell™400, STEMCELL Technologies) to form microspheroids with approximately 250 cells. The microspheroids were cultured in a chemically defined chondrogenic media (CM) for 4 days where after they were seeded onto MEW polycaprolactone (PCL) meshes with defined pore size. The PCL meshes were printed with Spraybase® Melt Electrospinning instrument with an average fiber diameter of 10.9 ± 2.3 µm and coated with 0.1% gelatin before seeding. Microspheroids on MEW-PCL meshes were differentiated in CM for an additional 14 days before gene expression analysis or subcutaneous implantation in immunodeficient mice was performed.

Results
Melt electrowritten polycaprolactone (MEW-PCL) meshes were tailored to contain pores with a size (116 ± 28 µm) designed to allow efficient entrapment of microspheroids (180 ± 15 µm in diameter). Four days microspheroids attached and spread over the MEW-PCL mesh. After 14 days in chondrogenic media, gene expression analysis demonstrated up-regulation of chondrogenic (9-fold: SOX9, 140-fold: COL2) and prehypertrophic (71-fold: IHH) gene markers. To assess the bone forming capacity of the "living membranes" (day 14), they were implanted subcutaneously with MEW-PCL meshes only as control. No mineralization was detected in

mesh-only explants but bone, bone marrow and mineralized cartilage was detected in all “living membranes”. A total amount of 23 ± 3 % (MV/TV) mineralized tissue was observed. These data demonstrated differentiation towards prehypertrophic chondrocytes in vitro and bone formation in vivo.

Discussion
The high versatility of this biofabrication approach lies in the possibility to tailor the scaffolds to shape and dimensions corresponding to the clinical indication and the individual patient using robust bone forming building blocks. We believe that these strategies will be instrumental in the development of designed living tissues with predictive clinical outcomes.

Acknowledgment
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Reference: 1. Nilsson Hall, G. et. al., Adv. Sci. 7, 1–16 (2020).

keywords: spheroids, endochondral ossification, melt-electrowriting

62825464088

LASER ASSISTED BIOPRINTING FOR SPHEROID-BASED TISSUE MANUFACTURING

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INTRODUCTION
In the field of bioprinting, which is growing very rapidly, the bioextrusion technology represents more than 80% of the market. The other technologies (e.g. microvalve or inkjet) are used for their ability to print droplets. Among them, laser-assisted bioprinting (LAB) represents the most advanced technology to achieve high resolution printing and high cell viability [1][2]. Poietis, a leading company in LAB technology, has developed a unique machine combining all bioprinting technologies in a single instrument to take advantage of their complementary performances paving the way toward personalized ATMPs for therapeutic applications [3]. In this context, bioprinting of spheroids is of great interest to produce efficacious tissue constructs for clinical applications [4][5]. The objective of this talk is to present the capabilities of the LAB approach to print spheroids in a reproducible manner while ensuring post-printing structural maintenance of spheroids and cell viability.

RESULTS
Spheroid LAB printing is possible by raising the laser energy to 25 µJ, the deposited volume to 30 µL and ablated gold surface to 7000 μm^2. First, a very thin, high speed jet appeared followed by a thicker jet at lower speed which is capable to transport the spheroid onto the receiver surface. These settings resulted in the formation of a jet with a diameter of 200µm and spheroid transfer success of 94%. The ultra-short laser was limited in the size of spheroid printing capability while the nanosecond laser gave a broader range.
Spheroids with different chondrogenic maturation were tested and day 7 ones exhibit the best printing efficiency possibly due to a trade-off in their composition (# cells, ECM) and size. Semi-quantification of viability staining demonstrated that printing using ultra-fast laser resulted in a similar viability as non-printed spheroids. Although the nanosecond laser resulted in a slightly lower cell viability after printing, the broader range of spheroid printability motivated its use for further experiments.
Furthermore, time resolved imaging technique enabled quantification of the jet dynamics to get experimental behavior laws for each condition. Next, numerical simulations contributed with physical interpretation to the experimental data.

DISCUSSION & CONCLUSION
We provide proof-of-concept for the use of LAB technology for spheroid-based tissue production, providing a reproducible and precise manner of transferring spheroids. A better understanding of the underlying physical processes and specific biological conditions have been obtained through dedicated experiments and simulations.

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keywords: Laser, bioprinting, spheroids, ATMP, Tissue Engineering

41883637086

DEVELOPMENT OF A ROBOTICS-DRIVEN BIOMANUFACTURING PROCESS FOR CARTILAGINOUS SPHEROIDS

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Introduction
For a viable and compliant clinical translation of tissue-engineered products, the adoption of automated technologies has been acknowledged as a prerequisite. Recently, the use of chondrogenic microtissue and organoid assemblies has shown promising results in long-bone defect regeneration through endochondral ossification[1]. Hence, automated biomanufacturing technologies able to culture and handle these tissue building blocks are of great interest. Here, we present an automation strategy through the use of different robotics for (i) media change during differentiation, (ii) plate movement, and (iii) image-based picking of microtissues for enabling spheroid-specific quality control.

Methods
Periosteum derived cells were seeded in a commercial microwell platform (AggrewellTM800, 1000 cells/spheroid or AggrewellTM400, 250 cells/spheroid). They were cultured for 21 days in chondrogenic medium. Media change of microtissues in microwell platforms requires controlled pipetting to avoid microtissue displacement and suspension leading to uncontrolled fusion. We set up a design of experiment (DoE) for aspiration and dispension speed during automated media changes. Then, we investigated the effect of robotic plate handling and automated imaging on spheroid movement for different size spheroids. After 21 days, we created ectopic implants through a controlled fusion of 900 large, or 3600 small spheroids to assess the bone-forming capacity, which is evaluated through µCT and safranin-O histochemistry. Subsequently, the automatic cell-screening and -picking system CellCelector™ was used to select and transfer single spheroids in a controlled manner. Here, spheroids were automatically selected based on the presence of only 1 spheroid/well and/or size via image-based analysis.

Results
The first DoE revealed that dispension speed, rather than aspiration speed, had a significant effect on local spheroid movement during media changes. The second DoE showed that smaller microtissues were more susceptible to movement than larger spheroids. Also, plate handling had a significant impact on overall movement. After 21 days, spheroids from all conditions were able to mineralize ectopically. Finally, using the CellCelectorTM, we were able to pick and place single spheroids without affecting their viability.

Discussion
Bottom-up engineering of skeletal implants requires a vast amount of diffusion-unlimited spheroids as building blocks. Culturing these for multiple weeks to achieve the desired differentiation is a complex process that requires expert personnel to avoid spheroid movement that leads to uncontrolled fusion. To enable scale-up and increase process robustness, we demonstrate the development of an integrated bioprocess for culturing and manipulation of cartilaginous spheroids. We anticipate the progressive substitution of manual operations with automated solutions for the manufacturing of microtissue-based living implants.

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keywords: Spheroid manufacturing, Robotics, Automation, Scale-up

20941861537

STIRRED CULTURE PROMOTES CHONDROGENIC HYPERTROPHY OF CARTILAGINOUS MICROTISSUES THROUGH EXPOSURE TO INTERMITTENT SHEAR STRESS

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Cartilage microtissues are promising tissue modules for bottom up biofabrication of implants leading to bone defect regeneration. Most of the protocols for the development of these cartilaginous microtissues have been carried out in static setups, however in order to further achieve higher scales suspension process needs to be investigated. In the present study we explored the impact of dynamic culture in a novel stirred microbioreactor system.

In order to generate cartilaginous microtissues, we first allowed cells to aggregate for 3 days, forming stable microtissues, before inoculating the bioreactors. Moreover a coupled CBM-CFD model was used in order to estimate the magnitude of shear stress on the individual microtissues. Experiments with the miniBR setup illustrated that we could culture cells dynamically in to form of microaggregates for up to 14 days. Furthermore, we observed that spheroids were able to fuse into larger structures , we used two static controls; one where spheroids cultured with the same dimensions as the initial inoculation and a second control where multiple spheroids were fused in to larger sized at the same time point as we inoculated the bioreactor. We observed that viability was not affected ether by the fusion event on (day2) or size of the microtissues while the same applied for culture in the bioreactor. Additionally, we saw a distinct difference between static and dynamic condition with a much lower fraction of EDU+ cells present in the bioreactor condition. This difference could be linked to the commitment of cells towards ECM production rather than proliferation something that is known during chondrogenic differentiation. This can be further evidenced by the histologic sections and Alcian Blue staining. Gene expression values showed a dramatic upregulation of both Indian hedgehog (IHH, 30-fold) and Collagen type X (COLX, 23-fold) well know marker of chondrogenic hypertrophy, for the day7 and day 14 time points for the dynamically cultured microtissues. Moreover Chondromodulin also showed a large upregulation (16-fold) for the last time point. Similarly to the transcriptome level, we saw distinct metabolic profiles between static and dynamic conditions. More specifically, whereas both conditions were characterized by high glucose consumption and lactate production at Day 7 and Day 14, in the bioreactor condition there was significant increase in glucose consumption and lactate production for Day 14 and a similar trend as well at Day 7. Moreover, several amino acids such as proline and aspartate showed significant differences between the conditions.

In this study we explored the impact of bioreactor-cultured microtissues in suspension as compared to statically cultured ones and the influence of shear stress on the acceleration of chondrogenic differentiation towards hypertrophy. Our work provides insights on the effect of the process environment on critical cellular, molecular, and metabolic parameters, and a straightforward strategy for the scalable production of cartilage intermediate microtissues.

keywords: bioreactors, developmental engineering, cartilaginous microtissues, chondrogenic differentiation

S65-1 Vascularization for Tissue Engineering and Regenerative Medicine Room: S1 (30 Jun 2022, 13:30 - 15:00)

Convener: Zygmunt Pojda

83871202505

THERAPEUTIC VASCULARIZATION IN REGENERATIVE MEDICINE

Andrea Banfi (Basel University Hospital, Basel, Switzerland)

Therapeutic angiogenesis, i.e. the generation of new vessels by delivery of specific factors, is required both for rapid vascularization of tissue-engineered constructs and to treat ischemic conditions. Vascular Endothelial Growth Factor (VEGF) is the master regulator of angiogenesis. However, uncontrolled expression can lead to aberrant vascular growth, as well as non-vascular side-effects. Major challenges to fully exploit VEGF potency for therapy include the need to control in vivo distribution of growth factor dose and duration of expression. In fact, the therapeutic window of VEGF delivery depends on its amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly bound to extracellular matrix. On the other hand, short-term expression of less than about 4 weeks leads to unstable vessels, which promptly regress following cessation of the angiogenic stimulus. Here we will briefly overview some key aspects of the biology of VEGF and angiogenesis and discuss their therapeutic implications, with a particular focus on approaches using gene therapy, genetically modified progenitors and extracellular matrix engineering with recombinant factors.

keywords: Angiogenesis, Mesenchymal Stem Cells, Ischemia, Bone Regeneration

94238147747

ENGINEERING HIGH DENSITY CAPILLARY-LIKE NETWORKS USING MICROPOROUS ANNEALED PARTICLE TISSUES

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INTRODUCTION
The vascular tree is essential for the function and survival of tissues. However, engineering vascular trees within 3D tissues has remained challenging. Current methods to produce channel-like structures in engineered tissues such as 3D printing are able to mimic large vessels, but struggle to produce highly dense capillary networks at high speeds, limiting their translation to clinically sized constructs.1 Microporous annealed particles (MAP) offer an interesting alternative due to their microporous nature, essentially offering an inherent dense microporous network of channels without having to create it.2 Here, we aim to use our recently developed in-air microfluidics (IAMF) technique to produce cell-laden microgels at ultra-high throughput production rates to create microporous annealed particle (MAP) tissues, allowing for the bottom up development of engineered tissues with inherent highly dense capillary sized pore networks.3

METHODOLOGY
Alginate-Tyramine (ATA) was produced by functionalizing alginate with tyramine groups using DMTMM-based coupling. Using IAMF, hepatic cell laden ATA microgels were prepared via ionic crosslinking under oil-free and surfactant-free conditions. ATA microgel slurries were photocrosslinked into MAPs using ruthenium, sodium persulfate and visible light. MAPs were analyzed on viability, micropore size distribution, pore interconnectivity, hydraulic conductivity, perfusability and mechanical properties.

RESULTS
Cell-laden microgels were successfully produced at varying cell concentrations, maintaining viability after MAP formation through visible light crosslinking. Moreover, MAPs were kept in culture for a period of 7 days and encapsulated cells proliferated within MAP constructs. Confocal analysis confirmed a highly dense, porous network within MAPs with the majority of pores having diameters below 40μm. Pores were shown to lead to increased construct perfusability as compared to nanoporous gels and an interconnected porous network was shown by perfusing 1μm fluorescent particles as well as using microCT analysis. Moreover, small molecules are able to easily perfuse in and out of the MAP as compared to traditional nanoporous gels that are hindered by the diffusion limitation.

CONCLUSION
The combined use of IAMF and ATA allows for ultra-high throughput production of cell laden microgels that can be assembled into MAP tissues containing an inherent, highly dense, interconnected capillary-like network. We thus present a novel and highly scalable production platform for the creation of large engineered tissues with inherent capillary-like networks.

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keywords: Microfluidics, bottom-up tissue engineering, vascularization, ultra-high throughput

62825408166

SEMAPHORIN3A COUPLES OSTEOGENESIS AND ANGIOGENESIS IN TISSUE-ENGINEERED OSTEOGENIC GRAFTS

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Introduction
Coupling of angiogenesis and osteogenesis is crucial to generate vascularized bone grafts. Semaphorin 3a (Sema3a) regulates osteoblasts and osteoclasts to promote bone synthesis through Neuropilin-1 receptor (NP1). We previously found that: 1) short-term delivery of Vascular Endothelial Growth Factor (VEGF) in osteogenic grafts dose-dependently decreases bone formation by increasing resorption and impairing progenitor differentiation; 2) in skeletal muscle VEGF dose-dependently inhibits endothelial Sema3a expression, impairing recruitment of Neuropilin-1-expressing monocytes (NEM), TGF-b1 production and SMAD2/3 activation. Here we investigated whether: a) VEGF impairs bone formation by inhibiting endogenous Sema3a expression; b) Sema3a treatment could improve both bone formation and vascularization in engineered bone grafts.

Methods
Fibrin matrices were decorated with recombinant VEGF or Sema3a proteins that were engineered with a transglutaminase substrate sequence (TG-VEGF and TG-Sema3a) to allow cross-linking into fibrin hydrogels. Osteogenic grafts were prepared with human bone marrow mesenchymal cells (BMSC) and hydroxyapatite granules in a fibrin hydrogel containing TG-VEGF, TG-Sema3a or both at ratio of 1:1 and implanted ectopically in nude mice. Sema3a blockade was achieved with a specific antibody (anti-NP1A) that prevents Nrp1 binding to Sema3a, but not to VEGF.

Results
100 mg/ml of TG-VEGF (high dose) caused severe bone loss and significant downregulation of endogenous Sema3a expression. 0.1 μg/ml of TG-VEGF (low dose), instead, preserved both bone formation and Sema3A expression. Loss-of-function experiments showed that blocking Sema3a/NP-1 signaling significantly impaired bone tissue development, increased osteoclasts recruitment and, interestingly, also decreased vascular invasion both in the absence and presence of low TG-VEGF. Further, Sema3a/NP-1 blockade significantly reduced both human progenitor survival and endogenous Sema3a expression, as well as phospho-SMAD 2/3 activation in both human progenitors and host endothelial cells. These data are consistent with a positive feed-back loop between Sema3a and TGF-b1 signaling, as we previously described in skeletal muscle. Conversely, in gain-of-function experiments, TG-Sema3a co-delivery was able to prevent bone loss induced by high TG-VEGF, while preserving efficient vascular growth. Notably, TG-Sema3a alone could increase both the amount of mineralized matrix and vascular invasion in the absence of any TG-VEGF.

Conclusion
These data suggest that Sema3a: 1) is required for intramembranous ossification in osteogenic grafts; and 2) provides a key molecular link coupling angiogenesis and bone formation. These data identify Sema3a as a promising target to generate vascularized bone grafts in a clinical setting.

keywords: Sema3a, VEGF, osteogenesis, angiogenesis, bone

73296308684

A BIOARTIFICIAL FIBRIN-BASED VASCULAR PROSTHESIS WITH A PRE-VASCULARIZED TUNICA ADVENTITIA

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Introduction
The ultimate goal in vascular tissue engineering is the generation of bioartificial blood vessels that resemble the morphology and function of native vessels as accurately as possible. Previous studies have shown that the tunica intima and tunica media of native blood vessels can be resembled in bioartificial vessels by applying physiological mechanical stimulation using pulsatile perfusion in vitro. However, until today only very few studies have focused on the integration of a functional tunica adventitia including a vascular network known as vasa vasorum, which are of pivotal importance for graft integration and nutrition. For this, it is not only essential to integrate a complex microvascular network in the outer layer of the prosthesis, but also to resemble the physiological longitudinal alignment of capillary vasa vasorum of native vessels. Here, we investigated the effect of physiological in vitro perfusion on the alignment of vasa vasorum capillaries in the tunica adventitia of small-diameter fibrin-based bioartificial blood vessels.

Methodology
Two-layered fibrin vessels were generated in a step-wise molding technique. First, acellular tunica media-equivalents were molded in a cylindrical mold. For this, a high-concentration fibrin matrix was generated using 25 mg/ml human fibrinogen resuspended in Medium 199. Polymerization was initiated by adding thrombin, factor XIII and Calcium chloride. After initial polymerization, compaction of the matrix was performed by centrifugation using a custom-built rotation device. The compacted tunica media-equivalent was then transferred into a second mold and the tunica adventitia-equivalent was molded around it. For cellularization of this layer, red fluorescent protein expressing human umbilical vein endothelial cells (EC) and adipogenous stem cells (ASC) were suspended in a low-concentration fibrin matrix. Consequently, segments were implemented in bioreactors and incubated for 72 h under longitudinal tension of 50% (tension alone), pulsatile perfusion with physiological cyclic stretch of 5% (pulse alone) at a frequency of 60 bpm, or both factors combined (tension+pulse). Control segments were incubated in cell culture tubes without mechanical stimulation (static).

Results
Complex microvascular networks were generated by endothelial cell self-assembly in the tunica adventitia of every group. Both, longitudinal tension and pulsatile perfusion induced physiological longitudinal alignment of vasa vasorum capillaries parallel to the main vessel axis. This effect was even more pronounced when both stimuli were applied simultaneously. Opposed to that, statically incubated controls showed randomly organized capillary networks.

Conclusions
Integration of a pre-vascularized tunica adventitia in bioartificial blood vessels is a promising strategy to facilitate early graft integration and immediate cell nutrition throughout the vessel wall after implantation of bioartificial vascular grafts. With longitudinal tension and cyclic stretch, we identified two mechanical stimuli that influence capillary tube orientation in tunica adventitia equivalents of fibrin-based bioartificial blood vessels in vitro. Thus, mechanical stimulation

represents an effective strategy to generate physiologically aligned vasa vasorum capillaries in cardiovascular tissue engineering.

keywords: vascular graft, mechanical stimulation, microvascular network, vascular tissue engineering, bioreactor technique

31412728567

LARGE SCALE FIBRIN-BASED TISSUE CONSTRUCTS SHOW CAPILLARIZATION UPON PERFUSION

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Introduction
Vascularization is crucial for proper implant survival and integration in the body. For an implant to be vascularized in vivo and thereby supplied with oxygen and the needed nutrients, the body's blood vessels have to invade the implant first. This is a very slow and often insufficient process, especially in thicker tissues, which can result in implant loss. A solution for this are pre-vascularized, fibrin-based implants that can be attached to the blood vessels in vivo, thereby giving a faster and more sufficient blood supply throughout the whole tissue construct enabling implant survival.

Methodology
We here present a bioartificial tissue construct with a fibrin matrix of 5 mg/mL fibrinogen concentration, containing red fluorescent protein-labeled human umbilical vein endothelial cells (RFP-HUVEC) and adipose tissue-derived stem cells (ASC), facilitating capillary-like network formation. Cellularized fibrin gels were either supported by enclosing them in a high-concentration fibrin capsule, or by walls of the custom built flow chamber. Two fibrin-based vascular grafts were integrated on each side of the construct as an in- and outlet for flow. These were interconnected with microchannels penetrating the cellularized matrix. After polymerization of the fibrin matrix, microchannels were endothelialized with RFP-HUVEC as well. Tissue constructs were then perfused for four days at a flow rate of 18 mL/min at 2-3 mmHg, with feeding medium containing VEGF, FGF, Ascorbic Acid, Aprotinin, TPA and reduced serum supplement.

Results
After four days of perfusion, homogenous capillary-like network formation throughout the cellularized fibrin matrix could be observed. Interestingly, cells between endothelialized microchannels started to align during capillary formation. Moreover, a radial alignment of cells around microchannels could be observed, with occasional sprouting of endothelial cells off cellularized microchannels.

Conclusion
By perfusion of low-concentration fibrin gels, vascularization of a tissue construct can be achieved. This offers the opportunity for the generation of various tissue constructs by introducing tissue specific cell types into the construct.

keywords: vascular tissue engineering, bioartificial organs, fibrin matrix, capillary networks

20941855928

RESET ENDOTHELIAL CELLS PROMOTE FETAL HEPATOCYTE MATURATION IN A 3D ORGANOTYPIC ENVIRONMENT

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Introduction
The lack of functional vascular networks is one of the most important hurdles in tissue engineering, limiting the obtainment of fully functional organs substitutes. Vascularization promotes efficient nutrients and oxygen supply, representing a key factor for in vivo application [1]. Endothelial cells not only act as the structural building blocks of vascular endothelium, but they also fulfil key functional tasks by interacting with parenchymal cells in a complex cellular interplay. In the context of liver bioengineering, recent works described enhanced hepatic maturation when combining endothelial cells with liver organoids [2,3]. 'Reset' vascular endothelial cells (R-VECs) have recently been identified as a promising candidate for graft re-endothelialization by demonstrating good adaptability and efficient colonization capacity in decellularized scaffolds [4]. The present work aims to combine R-VECs endothelial cells and human fetal liver organoids in decellularized ECM scaffold environment, to recapitulate the endothelial – parenchymal cells interplay.

Methodology
Human fetal liver organoids (post conception week, PCW = 5) were generated in Matrigel® 3D culture, after previous cells isolation via tissue disaggregation, and expanded in hepatic organoid expansion medium [5]. R-VECs were cultured on conventional tissue culture flaks supplied by endothelial cells growth medium (EGM2) medium. Mouse livers were cannulated via the portal vein and decellularized via already established detergent-enzymatic treatment [6]. 18 M cells were obtained from enzyme mediated organoid dissociation and were further seeded in decellularized scaffolds via portal vein injection. Repopulated livers were cultured in a custom-designed bioreactor in dynamic conditions provided by a peristaltic pump (flowrate = 3 ml/min) in hepatic organoid expansion medium for 7 days. Then, the same number of R-VECs were

seeded with the same seeding technique. Repopulated scaffolds were cultured in the bioreactor with hepatic organoid expansion medium supplied with Oncostatin-M and Dexamethasone [5], and EGM2 in a 1:1 volume ratio. Histological and immunohistochemistry analysis were performed to study scaffold repopulation and the expression of mature hepatic and endothelial markers. qRT-PCR analysis was performed focusing on hepatic maturation markers such as cytochrome 3A4,1A2 (CYP3A4, CYP1A2), hepatocyte nuclear factor 4 alpha (HNF4α) and alpha fetoprotein (AFP).

Results
Mouse livers were successfully decellularized as highlighted by complete translucent appearance. H&E analysis showed efficient scaffold repopulation after 14 - days dynamic bioreactor culture. Immunofluorescence staining revealed the presence of hepatic maturation markers (HNF4α, human albumin and Alpha1-anti-trypsin) in co-presence of endothelial markers (CD31, Von Willebrand factor), highlighting epithelial-endothelial cell interaction and re-arrangement. qRT-PCR results showed enhanced expression of HNF4α, CYP3A4 and CYP1A2 in the 3D dynamic culture compared to the static in vitro control. Accordingly, lower AFP expression was also evidenced with respect to control in vitro culture.

Conclusion
Endothelial cells are involved in key structural and functional tasks that have a pivotal role in building up of functional organ substitutes. In the present work, human fetal liver organoids demonstrated increased phenotypic maturation when cocultured with R-VECs in a 3D organotypic environment. Endothelial cells demonstrated to be key players for the achievement of functional hepatic tissue, boosting fetal stage hepatocytes towards a more mature phenotype.

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keywords: endothelial cells, liver organoids, endothelial-epithelial interaction, bioreactor, liver tissue engineering

83767265448

THE USE OF HUMAN SKELETAL MUSCLE MICROVASCULAR ENDOTHELIAL CELLS IN SKELETAL MUSCLE TISSUE ENGINEERING: FROM CELL ISOLATION TO IN VITRO PRE-VASCULARIZATION

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Introduction
One of the key challenges in the field of tissue engineering is the vascularization of tissue-engineered constructs. Until now, endothelial cells (ECs) derived from human umbilical cord have been the predominant EC type for the engineering of vascularized tissue. However, ECs of different origins display a great heterogeneity, reflecting in tissue- and organ-specific characteristics, which are important for interacting with surrounding cell types. Therefore, the use of skeletal muscle-specific microvascular endothelial cells (SkMVECs) may offer more potential for generating tissue-engineered muscle which mimic better the native muscle structure and physiology. Engineering a vascular network within an engineered tissue can be achieved by co-culturing ECs with myoblasts in a 3D co-culture setting, based on the capacity of ECs to self-assemble and form a vascular network. For this, it may be further beneficial to obtain both cell types from the same biological origin. In this work, we present the isolation of SkMVECs in combination with myoblasts from human skeletal muscle, followed by the investigation of an optimal culture medium for the co-culture of these two cell types. Finally, we demonstrate the application of SkMVECs for generating vascularized bio-artificial muscle.

Methodology
SkMVECs and satellite cell-derived myoblasts were isolated using an in-house developed protocol. Tissue was digested using an automated mechanical and enzymatic tissue dissociation procedure. Isolated single cells were cultured and separated using magnetic-activated cell sorting. Cell characterization was performed based on immunofluorescence staining and flow cytometry. Next, different media compositions varying in type and combination of growth medium and fusion medium were compared. The individual cell types were screened separately for behavior in each media composition. For SkMVECs, the formation of endothelial networks within a fibrin (1 mg/mL) hydrogel was evaluated. For myoblasts, the formation of multinucleated myotubes was assessed by performing a fusion assay. Finally, SkMVECs were applied for engineering co-culture bioartificial muscles as described in (1) using the explored culture media, and visualized using confocal microscopy.

Results
Isolated SkMVECs were found to express the endothelial-specific cell markers vWF and CD31. Isolated satellite cell-derived myoblasts were positive for desmin. The functional characteristics of the two cell types were tested and revealed endothelial network formation of SkMVECs on growth-factor reduced Matrigel, and the formation of multinucleated myotubes by isolated myoblasts. Next, two culture media consisting of a combination of serum-rich medium with a switch to serum-low medium after 3 days, were found to facilitate both a proper myotube and endothelial network formation. In a final step, the determined culture conditions were applied for the 3D co-culture of myoblasts and SkMVECs, and were demonstrated to facilitate the creation of a vascularized bioartificial muscle.

Conclusion
With the developed protocol, SkMVECs can successfully be isolated from human muscle biopsies. In addition, an optimal co-culture medium was identified which further allows the use of SkMVECs to tissue-engineer bioartificial muscles. This paves the way for the follow-up investigation of the vascular properties of SkMVECs and their potential for improving the physiological relevance of muscle tissue constructs.

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keywords: Skeletal muscle tissue engineering, myoblasts, microvascular endothelial cells, in vitro pre-vascularization

62825436244

CELL SHEET-BASED SKIN SUBSTITUTE TO MODULATE VASCULATURE AND INVESTIGATE WOUND-HEALING ASSOCIATED ANGIOGENESIS

Adele Mauroux (Silab, Brive, France), Sandrine Gofflo (Silab, Brive, Poland), Yoann Atlas (CIRB-Collège de France, Paris, France), Sylvie Bordes (Silab, Brive, France), Brigitte Closs (Silab, Brive, France), Ruggiero Florence (IGFL, Lyon, France), Laurent Muller (CIRB-Collège de France, Paris, France)

Vasculature plays an essential role in skin physiology and its architecture and function are altered in aged and diseased skin. There is thus a need to develop innovative 3D in vitro models with adjustable and amenable vasculature. Several in vitro skin models co-seeding endothelial cells with fibroblasts and keratinocytes have been proposed using scaffolds or bioprinting. However, they all fail in faithfully mimicking native skin microenvironment, including its complex ECM, papillary and reticular specificity or wound-healing associated microenvironment. Indeed, despite cellular remodeling of the scaffold material at the cellular level, exogenous proteins remain as the unique major component thus limiting assessment of the actual dermis microenvironment dynamics.

The aim of this work was to develop a vascularized skin substitute in a dermal microenvironment generated by fibroblasts and displaying plasticity in response to angiogenic factors or physiologic processes. We thus used the scaffold-free approach of cell sheet co-culture to produce the skin microenvironment. Skin primary fibroblast cell sheets co-seeded with endothelial cells or keratinocytes were cultured and superimposed to generate vascularized full-thickness skin substitutes. Using immunofluorescence and transmission electron microscopy, we confirmed the presence of a fully differentiated epidermis and well-structured dermal-epidermal junction. Whole-mount immunofluorescence demonstrated that endothelial cells organized into a dense vascular network throughout the dermis. Capillaries displayed a lumen and were stabilized by a basement membrane and the recruitment of perivascular cells. Modulating Vascular Endothelial Growth Factor (VEGF) concentration in the ng/ml range and time of application differentially affected angiogenesis in our model, resulting in distinct vascular network length and branching. Interestingly, these variations also impacted epidermis differentiation and proliferation. Furthermore, applying a full thickness wound to the skin substitute resulted in wound closure mimicking the time frame and ordered physiological process. In this context, we could follow centripetal revascularization by sprouting angiogenesis from the wound boundaries.

We have thus implemented a novel skin substitute displaying vascular plasticity in response to subtle angiogenic stimuli and wound healing. This model is of interest to mimic physiological and compromised skin conditions involving the vascular component (aging, neuro-inflammatory diseases, etc) and to evaluate the capacity of natural active molecules to restore skin vascular homeostasis.

keywords: skin vascularization, cell sheet culture, microenvironment, wound healing

S65-2 Vascularization for Tissue Engineering and Regenerative Medicine Room: S1 (30 Jun 2022, 15:30 - 17:00)

Conveners: Arnaud Scherberich

41883626205

PRO-ANGIOGENIC HYDROGELS FROM CELL-DEGRADABLE AND PHOTO-CURABLE ALGINATE

Julia Fernández-Pérez (Department of Complex Tissue Regeneration, MERLN Institute, Maastricht University, Maastricht, Netherlands), Antonio Feliciano (Department of Complex Tissue Regeneration, MERLN Institute, Maastricht University, Maastricht, Netherlands), Francis Morgan (Department of Complex Tissue Regeneration, MERLN Institute, Maastricht University, Maastricht, Netherlands), Lorenzo Moroni (Department of Complex Tissue Regeneration, MERLN Institute, Maastricht University, Maastricht, Netherlands), Mathew Baker (Department of Complex Tissue Regeneration, MERLN Institute, Maastricht University, Maastricht, Netherlands)

Angiogenesis, the process by which new blood vessels sprout from existing surrounding ones, is essential for the survival of implanted tissue engineered constructs. In fact, the lack of proper re-vascularization and core necrosis is the main pitfall of many developed biomaterials (1). In this study, we describe a highly tunable hydrogel system for the growth of capillaries based on alginate.

Alginate was chemically functionalized with norbornene. Norbornene forms a covalent bond with a thiol group in the presence of a photoinitiator upon exposure to UV light. The degree of functionalization obtained was 4,63%, as measured with NMR. Mechanical properties were analyzed by rheology and hydrogels ranged 10 – 1000 Pa, depending on final polymer concentration and concentration of cross-linker. Photoinitiator LAP was used at 2 mM. To introduce biodegradability to the system, we used matrix metalloproteinases (MMP)-cleavable sequences flanked by two thiol groups as cross-linkers. The speed of degradation can be tuned by modifying the sequence specificity to a range of MMPs. Non-degradable hydrogels were fabricated by using 2000 dalton PEG di-thiol. Degradable hydrogels dissolved when exposed to collagenase, while non-degradable gels remained unaltered.

In a one-pot-synthesis manner, alginate-norbornene, thiolated-RGD, dithiol MMP-cleavable peptide, cells, VEGF165 and photoinitiator were mixed. Solution was casted onto siliconized glass-slides and irradiated with UV for 30 seconds. Human Umbilical Vein Endothelial Cells (HUVECs) and Mesenchymal Stromal Cells (MSCs) were encapsulated in hydrogels in a 1:10 ratio (HUVEC:MSC). Cells were viable after cross-linking and were able to fuse and sprout, recapitulating the process of angiogenesis. Cell elongation was apparent already after 24 hours of culture. Hydrogels were stained against CD31 and imaged with confocal microscopy. Then, networks were analyzed and quantified using Amira and WinFiber3D (2). Vessel parameters were superior in hydrogels with lower degree of cross-linking, and thus softer, and with higher concentration of RGD. Initial studies have shown no major differences depending on the cell-binding peptide used: RGD (derived from fibronectin), YIGSR (a sequence found in laminin) and GFOGER (a collagen-derived peptide).

In conclusion, we have developed a platform that allows the study of the influence of stiffness, matrix degradability and ECM binding motifs on angiogenesis. Further work will focus on the use of these hydrogels as bioinks for 3D printing vascularized tissues and organoids.

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Protocols. 2020.

keywords: angiogenesis, alginate, hydrogel

20941842648

THERAPEUTIC EVALUATION OF A2-ANTIPLASMIN AS A HUMAN-DERIVED SUBSTITUTE TO THE FIBRINOLYSIS INHIBITOR APROTININ IN SURGERY AND REGENERATIVE MEDICINE

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Introduction
Fibrin biomaterial is widely used in the clinic as a tissue sealant and in pre-clinical research as a carrier material for growth factor delivery. In these applications, premature fibrin degradation leads to suboptimal tissue adhesion, recurrent bleeding and limited regenerative efficacy. Therefore, fibrinolytic inhibitors are commonly added into fibrin formulations, such as the potent inhibitor aprotinin. Nevertheless, the use of aprotinin in the clinic has been associated to some important side-effects, including some immunogenic reactions related to the bovine origin of the drug. In this project, we characterized the use of an endogenous human fibrinolytic inhibitor, α2-antiplasmin (α2PI), as a potential substitute for aprotinin. We first tested α2PI as an antifibrinolytic agent for applications in tissue sealants and for growth factor delivery in diabetic wound healing. Then, we assessed α2PI as a stand-alone hemostatic agent to reduce blood loss during surgery.

Methodology
We produced α2PI as a recombinant his-tagged protein in Human Embryonic Kidney (HEK) 293 mammalian cells and purified it by affinity-based chromatography. The longevity of α2PI-supplemented fibrin hydrogels was assessed in vitro in presence of plasmin, and in vivo upon subcutaneous implantation in mice using an in vivo imaging system (IVIS). Next, the delivery of angiogenic growth factors via α2PI-supplemented fibrin hydrogels was tested in a wound healing model in the db/db diabetic mouse and was quantified by histomorphometric analyses. More specifically, wound regeneration was assessed in terms of wound re-epithelialization, granulation tissue formation and wound angiogenesis. Finally, we used a tail vein bleeding model in mice to evaluate α2PI hemostatic properties upon intravenous injection.

Results
Incorporation of recombinant human α2PI into fibrin biomaterials significantly prolonged their duration in vitro and in vivo. Upon subcutaneous implantation, α2PI-supplemented fibrin implants remained present for over 30 days, thus vastly outperforming the implants supplemented by aprotinin. In the diabetic wound healing model, the delivery of angiogenic growth factors by α2PI-supplemented fibrin significantly enhanced granulation tissue formation and wound angiogenesis as compared to the delivery of growth factors in absence of α2PI. In addition, we observed positive trend toward enhanced wound re-epithelialization (p-value = 0.09). Finally, we demonstrated that α2PI had similar hemostatic properties than aprotinin in the mouse tail vein bleeding model in vivo, significantly reducing blood coagulation time and blood loss as compared to non-treated animals.

Conclusion
In conclusion, α2PI showed strong efficacy in vivo, both as an anti-fibrinolytic and as a hemostatic agent, therefore appearing as a highly competitive human-derived substitute

to the bovine aprotinin. Indeed, α2PI successfully increased the longevity of fibrin implants, enhanced growth factor delivery in diabetic wound healing and reduced bleeding time and loss upon intravenous delivery, respectively mimicking the 3 main applications of aprotinin in fibrin sealants, fibrin-mediated drug delivery and in surgery.

keywords: Fibrin biomaterials, protease inhibitors, wound healing, growth factors, hemostasis

41883639768

BLOOD VESSEL DETECTION ALGORITHM FOR TISSUE ENGINEERING AND QUANTITATIVE HISTOLOGY

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Immunohistochemistry and immunofluorescence for vascular network analysis play a fundamental role in basic science, translational research, and clinical practice. Due to their versatility and specificity, these techniques are also widely used in the tissue engineering context, where the study of neovasculature is crucial to assess the function of artificial tissue surrogates. However, identifying vascularization in histological tissue images is time-consuming and markedly depends on the operator's experience. This study introduces "Blood Vessel Detection – BVD", an automatic and ready-to-use morphometrical tool for quantitative analysis of vasculature in fluorescent histological images. BVD is based on the extraction and analysis of low-level image features and spatial filtering techniques, which do not require a training phase. The performance of the BVD algorithm was tested on four different datasets: a set of phantom images and on three sets of histological sections from three separate in vivo studies that specifically focused on the characterization of angiogenesis. The first study analyzed was an example of a rat abdominal wall defect treated by a polymeric patch loaded with microparticles able to release an angiogenic factor1. The second case was a rat infarction model treated with a bilayer biohybrid patch composed of polymer and extracellular matrix2. Finally, the algorithm was utilized to quantify angiogenesis in the case of ectopic organ regeneration3. Collectively, 173 independent images were analyzed, and the algorithm's results were compared to those obtained by human operators. The developed BVD algorithm proved to be a robust and versatile tool, quantifying the number, the diameters, the perimeters, the areas, and the spatial distribution of blood vessels within all the considered datasets. BVD is provided as an open-source application working on different operating systems. BVD is supported by a user-friendly graphical interface designed to facilitate large-scale analysis.

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keywords: Blood vessels, immunohistochemistry, image analysis, quantitative histology

83767214526

HOMING OF BONE MARROW MONONUCLEAR CELLS TO AXIALLY VASCULARIZED TISSUE ENGINEERING CONSTRUCTS

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Introduction
Inducing axial vascularisation of tissue engineering constructs is a well-established method to allow an adequate support of large 3-dimensional tissues. Progenitor cell chemotaxis towards axially vascularized tissues and its role in inducing neo-vascularisation and tissue regeneration has not been well characterized.

Methodology
In a prospective randomized controlled study including 32 male syngeneic Lewis rats we investigated the native capability of the axially vascularized constructs to specifically attract systemically injected bone marrow mononuclear cells (BMMNCs). The underlying mechanism for progenitor cell homing was investigated focusing on the role of hypoxia and the SDF1-CXCR4/7 axis. Sixteen animals were used as donors for BMMNCs. The other 16 animals were subjected to implantation of a tissue engineering construct in the subcutaneous groin region. These constructs were axially vascularized either via an arteriovenous loop (AVL, n=6) or via uninterrupted flow-through vessels (non-AVL, n=10). BMMNCs were isolated, labelled with quantum dots (Qdot® 655) and injected 12 days after surgery either via intra-arterial or intravenous routes. 2 days after cell injection, the animals were sacrificed and examined using fluorescence microscopy.

Results
The Qdot® 655 signals were detected exclusively in the liver, spleen, AVL constructs and to a minimal extent in the non-AVL constructs. A significant difference could be detected between the number of labelled cells in the AVL and non-AVL constructs with much more cells detected in the AVL constructs specially in central zones ($p < 0.0001$). The immunohistological analysis showed a significant increase in the absolute expression of HIF-1 in the AVL group in comparison to the non-AVL group. The PCR analysis also confirmed a 1.4-fold increase in HIF-1 expression in AVL constructs. Although PCR analysis showed an enhanced expression of CXCR4 and CXCR7 in AVL constructs, no significant differences in SDF1 expression were detected via immunohistological or PCR analysis.

Conclusions
At the examined time point, the AVL constructs were capable of attracting systemically injected BMMNCs in a mechanism probably related to a state of controlled hypoxia associated with a robust tissue formation.

keywords: axial vascularisation, progenitor cells, Chemotaxis

73296317367

THE IMPACT OF ENDOTHELIAL CELL YAP/TAZ ON NEO-ANGIOGENESIS IN BONE HEALING

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Introduction
Neo-angiogenesis describes the development of new blood vessels and takes place during bone healing in the fracture gap. Angiogenesis and the establishment of a functional vascular network is closely associated with bone formation and critical for scar-free healing. Type H vascular endothelial cells (ECs), characterized by high expression of CD31 and endomucin (Emcn) have been identified as key regulators for angiogenesis-osteogenesis coupling. However, the underlying mechanisms that drive revascularization, especially in the early phases from hematoma formation to cartilage development largely remain unknown. Here, we especially want to draw attention to the role of mechanosensors YAP/TAZ in ECs and how they affect angiogenesis and osteogenesis. By modulating YAP and TAZ in ECs we further want to unravel the different phases of angiogenesis during bone healing.

Methodology
We analyzed vessel ingrowth and organization under different fixation stabilities during bone regeneration. Female, 12 weeks aged mice with endothelial YAP/TAZ dKO were sacrificed 7 days or 14 days post-osteotomy, respectively. Soft callus formation, cell organization and ECM deposition within the fracture gap was analyzed by immunohistological protocols and second harmonic imaging (SHI). To characterize H type vessel formation, CD31 and endomucin antibodies were used. Confocal microscopy was employed to analyze the target proteins during the onset of neo-vascularization in bone regeneration. Endochondral ossification was analyzed in areas of bone healing and compared against areas in the growth plate.

Results
We could show that H type vessels (CD31hiEmcnhi) are present in the osteotomy gap, suggesting that they play a key role in de novo angiogenesis during pathological and regenerative processes. Further, we could show that H type vessels are surrounded by osterix expressing pre-osteoblasts, supporting their important role in angiogenesis-osteogenesis coupling during bone healing. Conditionally knocking out YAP/TAZ in ECs leads to an increase in bone vasculature in the hypoxic microenvironment of the osteotomy gap. Further, EC YAP/TAZ dKO induces more vessel crosses, suggesting that vessels fail to stabilize and build a functional basement membrane. Whereas in the pre-cartilage phase complete vascular invasion can be observed, vessels regress in areas of cartilage development in later stages. This suggests that two different mechanisms of angiogenesis exist during bone healing. Angiogenesis via endochondral ossification in the osteotomy gap shows similarities to the bone formation process at the growth plate, however, the latter being more organized.

Conclusion
Next to a downregulation of inflammation, successful bone regeneration requires angiogenesis. We explored here the role of EC mechanosensors YAP/TAZ and their ability to regulate the build-up of a capillary network. We could further show, that revascularization during bone healing occurs via two distinct pathways. Neo-angiogenesis at the pre-cartilage phase follows different mechanisms than angiogenesis via a cartilage template in later stages of bone healing. Understanding the mechanisms of angiogenesis and the effect of EC YAP/TAZ is essential to develop new therapies how to best accelerate bone healing, which would particularly important for the treatment of bone fractures in the elderly.

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Kusumbe, A. et al., Nature 507, 323–328 (2014)

keywords: YAP/TAZ, angiogenesis, bone regeneration, mechanobiology

62825402527

HUMAN IPSC BLOOD VESSEL ORGANOIDS AS A SOURCE OF FLOW-ADAPTIVE VASCULAR CELLS FOR TISSUE ENGINEERING OF PERFUSED MACROVASCULAR GRAFTS.

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Introduction
Tissue engineering of human blood vessels is pursued as a clinical revascularization therapy as well as to develop in vitro human blood vessel models with native-vessel like organization. Clinical revascularization strategies that use autologous vessels are hampered by poor quality and limited availability. Synthetic vascular scaffolds may be used as alternative, but for replacement of smaller vessels (<4 mm range) this is associated with high thrombogenicity and patency loss. Pre-cellularization of the polymer constructs with autologous vascular cells could protect smaller diameter grafts against patency loss and improve long term graft survival upon implantation. In vitro seeded vascular grafts using patient-derived induced pluripotent stem cells (iPSCs) could be used for disease modeling or therapeutic screening. Here, we use iPSCs vascular Organoid Derived Endothelial Cells (ODECs) and Mural Cells (ODMCs) as a (autologous) vascular cell source for in vitro tissue engineering of small diameter biodegradable vascular grafts.

Methodology
The 3D vascular organoid culture method described by Wimmer et al. [1] was further developed to isolate pure populations of ODECs and ODMCs, which can be cryopreserved and expanded in traditional 2D culture without loss in cell pool purity, viability, and proliferative capacity. 2D ODEC culture was used in dynamic flow and TEER experiments to determine the shear stress responsiveness of the cells. Both ODECs and ODMCs were subsequently seeded on 3 mm diameter degradable solution electrospun polycaprolactone-bisurea (PCL-BU) scaffolds and exposed to flow for 48 hours.

Results
The 2D dynamic flow experiments and TEER experiments showed that ODECs are shear stress responsive and were able to establish and restore the endothelial barrier after thrombin stimulation. Additional experiments demonstrated that ODECs and ODMCs could be successfully seeded in bilayer configuration on the PCL-BU scaffolds, forming the luminal endothelium and underlying medial layer that partially mimicked the layered structure of a human native vessel. Exposure of the resulting human organoid derived tissue engineered vascular graft (TEVGs)

to lumen perfusion in a flow-bioreactor setup showed integrity preservation of the bilayer configuration and endothelial attachment to scaffold substrate after subjection to flow.

Conclusions
In conclusion, iPSC derived vascular organoid cells can be successfully used as a source of functional, flow-adaptive vascular cells for tissue engineering of perfused macrovascular grafts. Therefore, our protocol offers a TEVG based solution for replacement of small caliber native human macrovessels using patient derived cells.

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keywords: vascular tissue engineering, organoids, endothelial cells, blood vessels

73296313124

THE EFFECT OF CARTILAGE MATURATION AND MINERALISATION ON ANGIOGENESIS DURING ENDOCHONDRAL OSSIFICATION

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Introduction
Endochondral ossification (EO) is the process of bone development via a cartilage template. It involves multiple stages, including chondrogenesis, mineralization and angiogenesis. Importantly, how angiogenesis contributes to EO is not fully understood. To characterise the interaction between human mesenchymal stromal cells(hMSCs)-derived cartilage and blood vessels, we designed an in vitro co-culture model comprised of tissue engineered hMSCs derived cartilage pellets that undergo mineralisation, and adipose-derived stromal cells (ASCs) and human umbilical vein endothelial cells (HUVECs) that form blood vessel structures. After characterisation of the pellets, we assessed the effect of their conditioned medium on angiogenesis using HUVECs migration and proliferation assays. Finally, we co-cultured the pellets, HUVECs and ASCs in a fibrin hydrogel to develop a comprehensive system to study cartilage vascularisation.

Methodology
Chondrogenic hMSC pellets (N = 3 donors) were generated by culture with medium containing transforming growth factor(TGF)-β3 (10ng/ml) for 28 days. For mineralized pellets, β-Glycerophosphate (BGP) (10mM) was added from day 7 and TGF-β3 was withdrawn on day 14. On days 7, 14, 21 and 28, conditioned media were produced by culturing the pellets for 24h in basal medium. Then, the pellets were harvested for gene expression and histological analysis. Thionine and Von Kossa stainings were performed to detect glycosaminoglycans (GAGs) and calcium deposits, respectively. Transwell migration and EdU-proliferation assays were employed to evaluate the effect of conditioned medium on HUVECs. To generate a 3D-vascular network, HUVECs and ASCs were simultaneously co-cultured with pellets in a fibrin hydrogel for 14 days. The vessel structures were visualised by immunofluorescent staining for laminin.

Results
Thionine and von Kossa staining evidenced successful in vitro cartilage formation and mineralisation, respectively. BGP exposure induced the formation of mineralised deposits which increased in time, while GAG staining progressively decreased. The mRNA expression of osteogenic (ALPL, IBSP) and angiogenic/remodelling markers (VEGFA, MMP13) in mineralised pellets showed the highest levels on d14 and decreased during late mineralisation. Transwell migration assays showed that conditioned medium from chondrogenic and mineralised pellets

stimulates HUVEC migration (24.2-folds and 16.8-folds vs. negative control, respectively). HUVEC proliferation was also increased after exposure to conditioned medium from chondrogenic or mineralised pellets (1.9-fold and 2-fold vs. negative control, respectively). Finally, by co-culturing pellets/ASCs/HUVECs in a fibrin hydrogel, we achieved the successful formation of a 3D vascular network. Confocal imaging analyses revealed contact between microvessels and chondrogenic/mineralised pellets.

Conclusions
In this study, we established an in vitro model of cartilage vascularisation during endochondral ossification. By characterising mineralising pellet cultures, we found that the expression of pro-angiogenic markers and the pro-migratory and pro-proliferative effects towards HUVECs are maximum during early mineralisation and then decrease. Furthermore, 3D in vitro vascular network formation was achieved in the presence of chondrogenic or mineralised pellets. Our in vitro 3D co-culture model can be applied for mechanistic studies on the role of angiogenesis in bone formation and repair, as well as disease modelling.

keywords: Endochondral ossification, vascularisation, mineralisation, in vitro model, mesenchymal stromal cells

31412735886

TOWARDS TISSUE-SPECIFIC VASCULARIZATION OF BIO-ENGINEERED SKELETAL MUSCLE CONSTRUCTS USING AUTOLOGOUS SKELETAL MUSCLE MICROVASCULAR ENDOTHELIAL CELLS

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Introduction
Prevascularization of tissue-engineered constructs before implantation is used to accelerate anastomosis with the host vasculature and thus to enhance successful implantation. To induce prevascularization, the co-culture of tissue-specific cells with endothelial cells is explored. To date, most co-culture studies are still conducted with human umbilical vein endothelial cells (HUVECs). However, this ultimately restricts clinical applications and thus ideally autologous cells are used. Furthermore, the mounting evidence of the endothelium as a regulator of regenerative processes in an organ/tissue-specific manner warrants the use of tissue-specific cells for tissue engineering. Skeletal muscle microvascular endothelial cells (SkMVECs) are an interesting candidate for prevascularizing tissue-engineered skeletal muscle as they are both autologous and tissue-specific. Here, we compare SkMVECs to HUVECs, both in 2D as well as in 3D bio-artificial muscles.

Methodology
Primary human SkMVECs were obtained from 3 different donors. These cells were thoroughly characterized in comparison to the current standard, HUVECs, through immunostaining and bulk RNA sequencing. Next, in vitro sprouting capacity of the SkMVECs was compared to the HUVECs using a conventional spheroid assay and tube formation assay. In addition, endothelial network formation in a fibrin hydrogel was evaluated over 14 days. And finally, direct and indirect co-cultures with human primary myoblasts were set up to evaluate the interaction between the SkMVECs and myoblasts.

Results
SkMVECs were extensively compared to HUVECs through bulk RNA sequencing to evaluate differentially expressed genes and these were annotated to the related pathways. Next, the angiogenic potential was evaluated and SkMVECs were found to sprout less compared to HUVECs in terms of sprout number but sprout length was found to be similar. Also, from the tube formation assay, a similar extent of tube formation was found but HUVECs were found to form tubes more rapidly. However, when both endothelial cell types were embedded in a fibrin hydrogel over a longer period, which is similar to our tissue engineering system, SkMVEC constructs were found to result in more branched endothelial networks compared to HUVECs. In addition, the more rapid proliferation of HUVECs compared to SkMVECs seemed to interfere with stable endothelial network formation after 10 days. Conditioned medium of both cell

types was used to dissect the cross-talk between myoblasts and endothelial cells and effect. Finally, to explore the potential of SkMVECs for skeletal muscle tissue engineering, 3D co-culture experiments with autologous myoblasts were performed to evaluate the interaction between the SkMVECs and myoblasts.

Conclusion
Taken together, SkMVECs are capable of forming stable, extensive endothelial networks in a relevant model for tissue-engineering applications. In contrast, the currently used standard endothelial cell type, HUVECs, was found to be highly angiogenic in short-term assays but less suited for long-term endothelial network formation. Furthermore, SkMVECs interact with autologous myoblasts and vice-versa, which further underscores their potential as a suitable endothelial cell source for the prevascularization of engineered skeletal muscle tissue.

keywords: Skeletal muscle tissue engineering, microvascular endothelial cells, myoblasts, co-culture

41883602164

GLUCOSE ENHANCES TRANSPLANTED MESENCHYMAL STROMAL CELLS FUNCTIONS PERTINENT TO ANGIOGENESIS

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INTRODUCTION
Mesenchymal stromal cells (MSCs) are potential candidates in tissue engineering applications. Nevertheless, MSC-based therapies have fallen short of the initial promise and hype due to the low MSC retention rate caused by the disruption of nutrient and oxygen supplies. Our previous studies established that the lack of glucose (but not oxygen) is fatal to human MSCs (hMSCs) because it acts as a pro-survival molecule for hMSCs upon transplantation. As a first step towards the engineering of a glucose releasing hydrogel, this study aims to provide insights into the effects of glucose on hMSCs paracrine function pertinent to angiogenesis in vitro and in vivo.

METHODOLOGY
In vitro experiment: Release of bioactive factors, angiogenic potential, and chemo-attractive potential of conditioned media (CM) from hMSCs towards human umbilical vein endothelial cells (HUVECs) were performed using Multiplex Luminex® Assays, Matrigel-Based Tube Formation Assay, and Incucyte® Live Cell Analysis System, respectively. The CM was obtained by exposure of hMSC to near-anoxia in the presence of glucose at 0, 1, or 5 g/L for 3 days. In vivo experiment: hMSC pro-angiogenic potential was investigated using hMSC-containing hydrogels loaded with either 0, 1, 5, 10, or 20 g/L glucose and hMSC-free hydrogels loaded with 20 g/L glucose, which were implanted ectopically in nude mice. The formation of new blood vessels was quantified using a micro-CT scanner after Microfil® injection at 21 days.

RESULTS
Glucose improves angioinduction of MSCs in near anoxia: Supernatant CM collected from hMSCs cultured with either 1 or 5 g/L glucose in near-anoxia for 3 days increased HUVECs migration and formation of vascular-like structure compared to that in the CM collected from hMSCs cultured without glucose. These data were corroborated by the increased amounts of pro-angiogenic factors (Angiogenin, VEGF-A, VEGF-C, Angiopoietin-1, Endostatin, and CCL2) in the CM collected from hMSCs cultured with glucose. Glucose improves the survival of MSCs and new blood vessels formation post-implantation: Upon ectopic implantation into nude mice, the volume of newly formed blood vessels within hMSCs-containing hydrogels loaded either 5, 10, and 20 g/L glucose exhibited a 2.4, 2.8, and 2.4 fold increase compared to hMSCs-containing hydrogels without glucose at days 21 post-implantation, respectively.

CONCLUSION
These data demonstrate that critical impact of glucose on MSC-mediated angiogenesis both in vitro under near-anoxia and in vivo using an ectopic mouse model. Further investigations are ongoing to determine whether endoplasmic reticulum stress is involved in the positive effects of glucose in MSC-mediated angiogenesis. All in all, the in vivo delivery of hMSCs in the presence of glucose strategy may be broadly helpful to improve not only the survival but also the angioinductive potential of hMSC-based therapies.

keywords: Mesenchymal stromal cells, Angioinduction, Angiogenesis, Regenerative medicine, Paracrine function

S66
Wanted: Dead or Alive? Quantitative microscopy of spheroid and organoid tissues
Room: S4 C
(30 Jun 2022, 11:00 - 12:30)

Conveners: Ruslan I. Dmitriev; Michael Monaghan

94355105055

INTRAVITAL MULTIPHOTON AND HIGHER HARMONIC GENERATION MICROSCOPY FOR VISUALIZING CELLULAR PROCESSES IN CANCER AND TISSUE ENGINEERING

Bettina Weigelin (Werner Siemens Imaging Center, University Tübingen, Tübingen, Germany)

Intravital microscopy (IVM) has revolutionized our understanding of single-cell behavior in complex tissues by enabling real-time observation of molecular and cellular processes in their natural environment. In preclinical research, IVM has emerged as a standard tool for mechanistic studies of therapy response and the rational design of new treatment strategies. For understanding immune function in the tumor microenvironment, higher harmonic generation, a label-free multiphoton imaging technique, provides tissue context and reveals guiding structures which steer immune cell migration and modulate T cell efficacy. The talk will highlight the role of IVM for understanding cellular interactions with implanted materials and immune cell behavior in tumors within tissue-engineered bone.

keywords: Intravital multiphoton microscopy, higher harmonic generation, cancer, immunotherapy, bone tissue engineering

73296360669

NON-INVASIVE CLASSIFICATION OF MACROPHAGE POLARISATION BY 2P-FLIM AND MACHINE LEARNING

Nuno Neto (Trinity College Dublin, Dublin, Ireland), Sinead O'Rourke (Trinity College Dublin, Dublin, Ireland), Mimi Zhang (Trinity College Dublin, Dublin, Ireland), Hannah Fitzgerald (Trinity College Dublin, Dublin, Ireland), Aisling Dunne (Trinity College Dublin, Dublin, Ireland), Michael Monaghan (Trinity College Dublin, Dublin, Ireland)

In this study, fluorescence lifetime imaging of NAD(P)H-based cellular autofluorescence is applied as a non-invasive modality to classify two contrasting states of human macrophages by proxy of their governing metabolic state. Macrophages were obtained from human blood-circulating monocytes, polarised using established treatments, and metabolically challenged using small molecules to validate their responding metabolic actions in extracellular acidification and oxygen consumption. Fluorescence lifetime imaging microscopy (FLIM) quantified variations in NAD(P)H-derived fluorescent lifetimes in large field-of-view images of individual polarised macrophages also challenged, in real-time with small molecule perturbations of metabolism during imaging. We uncover FLIM parameters that are pronounced under the action of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) which strongly stratifies the phenotype of polarised human macrophages. This stratification and parameters emanating from a FLIM approach, served as the basis for machine learning models. Applying a random forest model, identified three strongly governing FLIM parameters, achieving a ROC AUC value of 0.944 when classifying human macrophages. Visualisation of the data show a clear classification of IFNγ-M1 and IL-4-M2 macrophages in response to real-time imaging when treated with FCCP. The excellent performance of machine learning models, applied on the data extracted from the non-invasive technique, underlines further the efficiency of this workflow. This workflow can be easily adopted to non-invasively characterise macrophage polarisation in in vivo models and in vitro multicellular organoid models to study foreign body interactions, biomaterial assessment, pharmaceutical research and screening and clinical applications such as disease diagnosis. Precise regulation of macrophage activation state is key to understanding disease control, tissue homeostasis and implant response, with this regulation shown to be directly related with macrophage intracellular metabolism. Therefore, impaired macrophage metabolism results in impaired function such as the case of diabetes, the foreign body response to biomaterials, obesity or cancer.

keywords: FLIM, Imaging, macrophages, non-invasive, microscopy

41883616084

MONITORING OF LIVE SPHEROID OXYGENATION USING FLUORESCENCE MICROSCOPY AND NANOSENSORS

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Introduction
Multicellular spheroids can be a powerful model mimicking the physiological environment of the tissue in a microscale format. They are often seen as 'microscale-bioreactors' providing an appropriate environment for cell differentiation and stem and cancer cell niche, which makes them important for physiological studies and biofabrication. However, this advantage becomes a problem when it comes to the question of characterization / standardization of spheroids produced in different labs, by different methods or even cell passage number1. The gradients of cell proliferation, oxygenation, cell death and other biomarkers are thus largely unexplored and ignored.

Methodology
Here we describe a nanosensor-based analysis of live spheroid oxygenation helping to routinely estimate O2 gradients on a conventional fluorescence microscope. Described method helps spheroid phenotype characterization (size, relative hypoxia), informing on their metabolism and viability. We optimized generation of spheroids loaded with ratiometric red (650 nm reference) / near infrared- (760 nm O2-sensing channel) emitting O2-sensing biocompatible nanoparticles. Presented visualization of oxygenation can be combined with multiparametric analysis in available 'blue' and 'green' channels such as cell death staining or advanced imaging modalities such as FLIM and PLIM2.

Results
Presented approach was tested in different experiments with homo- and heterocellular (human dental pulp stem hDPSC with endothelial HUVEC cells) spheroids produced from stem and cancer cells: (1) we performed long-term monitoring of individual spheroids oxygenation for more than 14 days; (2) we detected changes in oxygenation upon adding mitochondrial uncouplers / inhibitors; (3) we performed 'endpoint' multiparameter analysis of oxygenation coupled with labeling cell death by SYTOX Green. To standardize the analysis of oxygenation in spheroids we looked at oxygenation at spheroid core and periphery, value of O2 gradient and their 'steepness'.
We found that in contrast to hDPSC spheroids, 'addition' of HUVEC cells to spheroids provided higher oxygenation and significantly steeper gradient. Heterocellular spheroids were also statistically larger, suggesting that their oxygenation was caused by cell composition-related differences in bioenergetics agreeing with the known data on HUVEC and hDPSC metabolism.
To illustrate the applicability of the approach for biofabrication we compared O2 gradients in hDPSC spheroids before and on a day 1 after bioprinting in GelMA. Bioprinted hDPSC spheroids had significant changes in periphery which affected the range and steepness of their periphery-to-core O2 gradients. The dead cell staining was more profound in bioprints.

Conclusions
We demonstrated that spheroid oxygenation reflects the bioenergetic state and viability of cells in 3D, allowing application of ratiometric oxygenation analysis for standardization of spheroid phenotype. Usage of ratiometic analysis versus phosphorescence lifetime calculation enables for more 'cost-efficient' O2 gradients studies with almost all types of conventional fluorescence microscopes. The method is compatible with multi-parameter physiological measurements (e.g., cell death, proliferation, and cell composition) and downstream assays (immunofluorescence, FACS etc.), and long-term monitoring, essential for bioprinted constructs containing spheroids.

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keywords: oxygenation, spheroids, O2 probe, fluorescence microscopy, ratiometric fluorescence, nanosensors, near infrared

62825437764

INTEGRATED IMAGING AND MODELLING OF ORGANOID AND SPHEROID MORPHOMETRY USING SMART ALGORITHMS

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Introduction
Advanced in vitro models (e.g., organoids) are three-dimensional (3D) constructs usually generated from cells with a degree of stemness. This, accompanied by the multiplicity of parameters which condition organoid growth and morphology, results in constructs of different shapes and size and thereby functional properties. To date, a quantitative framework for robust measurement and modelling of organoid growth processes in relation to morphological features is still lacking and urgently required. In silico methods integrate physical data and biochemical models using computational tools, and are a powerful support for tissue engineering. They are particularly relevant for studying organoids: the multiplicity of parameters which condition their growth and morphology can be explored in virtual models, facilitating experimental design, and enabling prediction and extrapolation of behaviour and function. Here we describe a framework for quantifying organoid morphometry with imaging tools and mapping shape and size to virtual organoids generated through evolutionary algorithms.

Methodology
Multi tissue hepatic organoids and spheroids are generated using standard protocols. They are imaged at multi-scale by means of an integrated approach involving light-sheet, confocal and super-resolution microscopy. Thus, we can resolve objects spanning from few millimetres (e.g., construct shape) down to microns (e.g., cells) to tens of nanometers (e.g., mitochondria, tight junctions). Ad hoc image processing algorithms and routines are developed for dealing with the acquired datasets. In particular, algorithms based on the intensity distributions of background and foreground, described locally within the dataseta, are exploited for identifying and isolate single cells and/or subcellular constructs.
Evolutionary algorithms based on the optimization of a cost function which incorporates resource uptake, surface energy and cooperative metabolic effort are used to generate virtual organoids within a range of masses.

Results
Quantitative descriptors to characterize construct and cell shape, cell arrangement in the 3D space as well as cell-cell and cell-substrate interactions are identified. Evolutionary algorithms are honed by matching imaging data with the virtual organoids. We are thus able to identify how resource assimilation and physical phenomena affect organoid formation and growth.

Conclusions
Our framework enables the characterisation of structural features of 3D constructs, which in turn may give insights on their functionality. In addition, the outputs are used for implementing more accurate computational models that take into account the real shape of the constructs and the real arrangement of the cells. This will serve to quantitatively assess to what extent organoids are similar to their in vivo counterparts, and thus define strategies for improving reproducibility and viability. Integrating experimental and modelling approaches is key for designing constructs with translational value and hence useful for robust in vitro to in vivo

extrapolation, paving the way towards predictive and precision medicine and reducing animal tests. Currently the models are deterministic, future efforts will be dedicated to incorporating fluctuations as an inevitable and ubiquitous feature of any functional biological system.

keywords: imaging, evolutionary algorithms, morphometry, organoid, spheriod

41883615759

EMT TRANSCRIPTIONAL RESPONSE ARE TRIGGERED IN RESPONSE TO LASER PHOTOABLATION IN 3D MODELS OF MELANOMA

Daniel Rodrigues (3B's Research Group, Guimarães, Portugal), Daniela Moreira (3B's Research Group, Guimarães, Portugal), Cláudia Gomes (3B's Research Group, Guimarães, Portugal), Rui Reis (3B's Research Group, Guimarães, Portugal), Rogério Pirraco (3B's Research Group, Guimarães, Portugal)

Introduction
Lasers have been used for years in the field of cancer therapy. Nanosecond pulsed lasers, in particular, have been used in the generation of reactive oxygen species via plasma for the triggering of immunogenic cell death[1][2], for the delivery of biomolecules intracellularly via optoporation[3] and for tumor resection[4]. Notwithstanding this large range of uses, the full impact of nanosecond pulsed lasers on cellular mechanisms is not fully understood. Their effects on epithelial to mesenchymal transition (EMT), for instance, are unknown. While being a key mechanism in embryogenesis and wound healing, EMT has also been associated to tumor progression, invasion and resistance through the action of players like the transforming growth factor beta (TGF-β) family.
Here, the putative effects of nanosecond pulsed laser ablation on the expression of known EMT players in melanoma spheroids models are studied.

Methodology
Multicellular spheroids comprising the human melanoma cell line VMM-15 and human dermal fibroblasts (hDFbs) were produced while using monoculture spheroids of each cellular type as controls. Cellular aggregation was allowed to occur over 7 days, at which time partial ablation of the spheroids using a nanosecond 355nm laser was performed. Ablated spheroids were collected after allowed to recover for 3 hours or 3 days. Several key properties of the ablated spheroids where subject to evaluation. Morphology recovery was assessed using time-lapse microscopy. Gene and protein expression were analyzed by Real-Time PCR, Western Blot and immunohistochemistry.

Results
Ablated spheroids displayed variable angular openings of the wound surface, with greater values being verified for the multicellular spheroids. Laser ablation triggered an up-regulation of the gene expression of EMT mediator TGFβ1 across all conditions, which was also verified for its receptor TBFβ-R1. Canonical pathway transducer pSMAD2/3 presented a higher protein expression post-ablation. Additionally, increased gene expression was verified for PLOD2 which correlated with the increase of Col1 protein confirmed through western-blot. Further analysis of EMT players showed a decrease in the expression of epithelial marker E-Cadherin after ablation recovery, while an increase in the expression of mesenchymal marker N-Cadherin and ZEB1 was observed when compared to control. Gene expression of stem cell master regulators SOX2, OCT4 and NANOG was shown to be overexpressed in melanoma spheroids after laser ablation.

Conclusions
It was demonstrated that laser ablation triggers a phenotypical shift from an epithelial state into a mesenchymal state in melanoma cell spheroids mediated by the expression of TGF-β1. This was further reflected in the increased expression of key stem cell regulators linked to stemness. These results strongly suggest that nanosecond pulsed laser ablation is capable of promoting EMT in determined conditions.

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keywords: Spheroids, Cancer, EMT, Photo-ablation, Melanoma

S67
We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration
Room: S4 A
(30 Jun 2022, 13:30 - 15:00)

Conveners: Marianna Tryfonidou; Lizette Utomo

83871201684

A BIOMIMETIC APPROACH TO REGENERATE A FUNCTIONAL NP TISSUE IN THE DEGENERATING INTERVERTEBRAL DISC

Keita Ito (Eindhoven University of Technology, Eindhoven, The Netherlands)

The development of regenerative therapies for the intervertebral disc (IVD) is of much interest because IVD degeneration is a major cause of low back pain, one of the leading causes of disability worldwide. The most common approaches are to utilize biomaterials, cells, and/or molecular agents, alone or in combination. Some of these approaches are moving toward clinical implementation and have shown promising clinical results. However, actual tissue regeneration has been more elusive.

Tissue engineering and regenerative medicine have often focused on tissue morphology but clinically tissue and organ function may be more important to reduce pain and disability. In terms of the IVD, whose function is mainly biomechanical as a cartilaginous intervertebral joint, this has not always been the focus when designing regenerative approaches. In our and partner laboratories, we have taken a biomimetic approach to develop biomaterials that help to return IVD function. This talk will explain some of these approaches, how they have been evaluated and highlight some major challenges experienced early in their clinical implementation.

keywords: intervertebral disc, biomaterials, biomimetic, functional regeneration

31451703426

DEVELOPMENT OF ADVANCED REGENERATIVE APPROACHES FOR DISC DEGENERATION - CONSIDERATION OF THE DEGENERATE NICHE

Christine Le Maitre (Sheffield Hallam University, Sheffield, United Kingdom)

Low back pain is the leading cause of morbidity worldwide and yet most therapies fail to target the cause and are purely symptomatic or end stage surgical options. Intervertebral disc degeneration is associated with approximately 40% of low back pain cases and thus a target for potential regeneration. Intervertebral disc degeneration is a catabolic process caused by altered cell behaviour and tissue biomechanics, leading to a harsh environment for potential cell therapies. To generate a successful regeneration strategy for the intervertebral disc this harsh environment must be considered, and therapies assessed within conditions which mimic this degenerate niche.
The utilisation of cells alone for regenerative therapies are unlikely to be successful if the degenerative cascade and mechanical environment are not restored, hence the combination of cells with biomaterials offers advanced therapeutic approaches. Injectable biomaterials which can restore the mechanical properties of the disc, inhibit catabolic processes of disc degeneration whilst delivering a regenerative cell source hold the most promise to halt disc degeneration and enabling regeneration.
Here, the development of novel injectable hydrogel systems which show potential to deliver a three-pronged attack to regeneration of the disc will be discussed. The application of differential cell sources including mesenchymal stromal cells from adipose and bone marrow, notochordal cells and induced pluripotent stem cells will be discussed. Importantly model systems which can recapitulate the degenerate disc environment as testing platforms for potential regenerative approaches for the disc will be introduced.

keywords: Intervertebral disc, Regeneration, Injectable hydrogels, Regenerative cells

31412708286

TARGETED PROTEOMIC ANALYSIS TO EXPLORE THE ANTI-INFLAMMATORY EFFECTS OF NOTOCHORDAL-CELL DERIVED MATRIX

Frances Bach (Utrecht University, Utrecht, Netherlands), Lisanne Laagland (Utrecht University, Utrecht, Netherlands), Frank Riemers (Utrecht University, Utrecht, Netherlands), Adel Medzikovic (Utrecht University, Utrecht, Netherlands), Gerrit Erdmann (NMI TT Pharmaservices, Berlin, Germany), Laura Creemers (University Medical Centre Utrecht, Utrecht, Netherlands), Christophe Sachse (NMI TT Pharmaservices, Berlin, Germany), Keita Ito (Eindhoven University of Technology, Eindhoven, Netherlands), Markus Templin (NMI TT Pharmaservices, Berlin, Germany), Marianna Tryfonidou (Utrecht University, Utrecht, Netherlands)

Introduction
Low back pain due to intervertebral disc (IVD) degeneration is a major health and socioeconomic problem throughout the world. In the young and healthy IVD, large and vacuolated notochordal cells (NCs) are present1. These cells are, in some species (e.g. humans and dogs), replaced by chondrocyte-like nucleus pulposus cells (NPCs) during maturation and ageing2. In previous studies, porcine NC-derived matrix (NCM), containing matrix and biologic factors secreted by NCs, induced regenerative and anti-inflammatory effects in human, canine, and bovine NPCs in vitro and degenerated canine IVDs in vivo3,4. Since the precise mechanism behind NCM remained elusive, we aimed to determine the mode of action of NCM in the degenerative IVD environment.

Methodology
Canine and human NPCs were cultured with and without NCM for 6, 24, and 72 hours in monolayers. After 6, 24, and 72 hours, RT-qPCR was performed on inflammatory markers and after 72 hours, targeted proteomics was performed with DigiWest, a proprietary immunoassay technology which transfers Western Blot to a high-throughput bead-based microarray platform5. Lastly, immunohistochemistry was performed on in vivo canine IVDs treated with NCM for 6 months.

Results
RT-qPCR analysis indicated that NCM induced an initial inflammatory response after 6 hours, since IL-6, IL-8 and COX2 mRNA expression was increased in human and canine NPCs. DigiWest analysis showed that NCM mainly induced changes in the Mitogen-activated protein kinase (MAPK) pathway after 72 hours of treatment, i.e. after the initial pro-inflammatory response. The expression of key proteins downstream the MAPK pathway, such as ERK1/2, JNK, and PKC, was mostly inhibited by NCM, whereas expression of proteins that are known to dephosphorylate MAPK key signaling molecules, DUSP1, 5, and 6, was increased in NCM-treated NPCs. Lastly, also expression of KRT19, a healthy NP marker, was induced by 72 hours of NCM treatment. Confirming the DigiWest results, in vivo canine IVDs treated with an intradiscal NCM injection demonstrated increased KRT19 and DUSP5 immunopositivity compared with controls after 6 months of treatment.

Conclusions
Taken together, these results indicate that NCM induces an initial inflammatory response, but thereafter exerts its prolonged anti-inflammatory effects by influencing the MAPK pathway. The latter leads to reduced expression of inflammatory cytokines after prolonged treatment.

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keywords: notochordal cell, inflammation, proteomics, animal model

31412709597

MODIC CHANGES CORRELATE WITH ENDPLATE AND VERTEBRAL BONE PATHOLOGIES IN DOGS

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Introduction
The last decade, efforts have been made in developing more effective diagnostics for low back pain. The focus was addressed towards Modic Changes (MCs), pathological signal intensity changes in the vertebral bone marrow and endplates of the intervertebral disc (IVD), which can be detected on Magnetic Resonance Imaging (MRI)1. In contrast to the human situation, little is known about the prevalence and characteristics of MCs in dogs, that are often used as a model to study intervertebral disc disease and new regenerative therapies2. For this reason, the aim of this study was to examine the prevalence and imaging/histologic characteristics of MCs in dogs.

Methodology
High field 1.5 Tesla MRI images of canine patients with low back pain and/or neurological deficits were retrospectively analysed. Inclusion criteria were the availability of sagittal T1- and T2-weighted turbo spin-echo sequences for the whole lumbar spine and exclusion criteria were (para)spinal neoplasia, resulting in 340 dogs and 2496 spinal segments. Dogs that underwent necropsy on the same day of the MRI were used for histo(patho)logical analysis (modified Boos score3, including endplate morphology, new bone formation, and subchondral bone sclerosis; n=16 dogs, 39 segments). The adjacent vertebral bone was assessed for infiltration of inflammatory cells, neovascularization, fatty infiltration, and the presence of chondroid cells, fibrous tissue, or Schmorl's nodes. Multivariable logistic regression models were built to test the association between the presence of MCs and explanatory variables.

Results
MCs were most often detected at the lumbosacral junction (L7-S1 in the dog), the majority was MC type 3 (subchondral bone sclerosis). Previous spinal surgery at the investigated level predisposed dogs for the development of MC type 1 (proliferation of fibrovascular (granulation) tissue, oedema in vertebral bone marrow) and 2 (fatty infiltration). As in humans, MCs in dogs were interconvertible over time. The prevalence of MCs appeared positively associated with age and disc protrusion/extrusion. Lasty, histological analysis indicated that IVDs in which MCs were detected showed more histopathological changes in the endplate and vertebral bone than IVDs without MCs. However, the histological changes described in human literature were not detected in the segments with the specific MC types. Instead, mostly chondroid infiltration was encountered in MC types 1 and 3.

Conclusions
As humans, also dogs show MCs, mostly at the lumbosacral junction. However, MCs in dogs exhibit other subchondral bone pathologies than humans, as chondroid proliferation was mostly encountered and little proliferation of fatty, fibrous, or granulation tissue.

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The European Union's Horizon 2020 program (iPSpine; #825925) supported this work.

keywords: modic changes, dog, histology, intervertebral disc

52354506186

DIRECTED DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TO NOTOCHORDAL-LIKE CELLS BY COMBINATORIAL TRANSCRIPTION FACTORS ACTIVATION

Xiaole Tong (Utrecht University, UTRECHT, Netherlands), Deepani Poramba-Liyanage (Utrecht University, UTRECHT, Netherlands), Peng Shang (Leiden University Medical Center, Leiden, Netherlands), Niels Geijsen (Leiden University Medical Center, Leiden, Netherlands), Danny Chan (The University of Hong Kong, Hong Kong, China), Anne Camus (Université de Nantes, Nantes, France), Marianna Tryfonidou (Utrecht University, UTRECHT, Netherlands)

Introduction
Low back pain (LBP) is a leading cause of disability worldwide and intervertebral disc (IVD) degeneration (IVDD) is a major contributor of LBP1. The IVDD is accompanied and often preceded by the replacement of large vacuolated nucleus pulposus cells (NPCs) by non-vacuolated, clustered cells in the notochordal cells (NCs) of the IVD2. The iPSpine project aims to re-populate the degenerated IVD with regenerative iPS-derived NC-like cells (iPS-NLCs). During embryonic development, the determination of cell fates is a result of the combinatorial and concomitant activation of transcription factors. While differentiation of iPS-NLCs can be partially achieved by mRNA transfection of a single gene, such as the notochord-related transcription factor NOTO, such attempts are limited by low and variable differentiation efficiency3. This study aims to achieve optimal notochordal lineage commitment by the concomitant and combinatorial activation of multiple key transcription factors via CRISPR activation (CRISPRa) technology. With CRISPRa, transcription activation complexes are recruited to the endogenous promoters of genes to induce expression.

Methodology
Based on a two-step differentiation protocol, we first established iPS-derived mesodermal progenitor cell by CHIR stimulation to activate the WNT pathway. For notochordal lineage commitment, we focus on NOTO, brachyury (TBXT) and Forkhead Box Protein A2 (FOXA2). Both T and FoxA2 act upstream and are required for the NOTO expression. These three genes were activated at the endogenous gene locus by CRISPRa technology via the synergistic activation mediator (SAM) system, the most efficient dCas9 gene activator4. Gene activation by CRISPRa will be further compared with mRNA transfection to establish the optimal mode of notochordal lineage commitment.

Results
We establish a gene activation and differentiation pipeline in the notochordal cell lineage and show significant activation of all 3 genes by recruitment of CRISPRa to the respective gene promoters in the differentiating iPSCs. Our results indicate better expression levels when transcription is promoted directly via recruitment of transcriptional activation complexes to gene promoters, rather than the introduction of synthetic mRNAs. Time-course analysis of lineage-specific markers shows that combining multiple transcription factors allow for better iPSC-NLC differentiation and commitment toward the notochordal lineage.

Conclusions
We highlight how transcriptional landscapes can be modulated at critical moments of embryonic development to optimize iPSC-NLC differentiation strategies and optimize notochordal lineage commitment.

Funding: iPSpine: Horizon2020(No.825925) and Dutch Arthritis Society (LLP22).

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keywords: iPSC differentiation; intervertebral disc; CRISPR activation; transcription factor activation

52354503848

TUNING THE PHYSICAL PROPERTIES OF COLLAGEN/HYALURONAN HYDROGELS TO FAVOR MESENCHYMAL STEM CELLS DIFFERENTIATION INTO NP CELLS: A STEP FORWARDS INTERVERTEBRAL DISC REGENERATION

Antoine Frayssinet (Sorbonne University - CNRS, Paris, France), Esther Potier (Université de Paris - CNRS, Paris, France), Gervaise Mosser (Sorbonne Université - CNRS, Paris, France), Matteo D'Este (AO Foundation Institute, Davos, Switzerland), Christophe Helary (Sorbonne Universite - CNRS, Paris, France)

Back pain is often associated with intervertebral disc (IVD) degeneration. Beside surgery, novel treatments relying on stem cell injection have been tested. Unfortunately, the outcomes are disappointing because of cell leakage and incomplete differentiation. Nowadays, a consensus exists on the necessity to encapsulate stem cells within a hydrogel to maintain them in situ and favor their differentiation. As cell behavior depends on biochemical and physical environment, a biomimetic hydrogel would promote IVD regeneration. Nucleus Pulposus is a highly hydrated tissue working as hydraulic shock absorber. Glycosaminoglycans give a high degree of hydration whereas collagen II gives resistance and allows for cell adhesion. With the aim of developing novel biomimetic hydrogels, collagen/hyaluronic acid composites were developed to mimic the structure and the mechanical properties of Nucleus Pulposus. For this purpose, we first studied the impact of the HA content on physical properties. Then, the potential of the different formulations to differentiate mesenchymal stem cells (MSCs) into NP cells was analyzed in detail.

HA functionalized with tyramine groups (HA-Tyr) was mixed with collagen and gelled using Horse Radish Peroxidase and H2O2 at pH 7.4. With a constant collagen concentration (0.4%), the HA-Tyr content was increased up to 2 % to create a platform of Col/HA hydrogels with different properties. The hydrogel structure, the mechanical properties and the degree of hydration were analyzed. Mesenchymal stem cells were encapsulated within the different hydrogel types and cultivated over 28 days. The impact of MSCs on hydrogel stability, metabolic activity and cell morphology were analyzed. Last, the gene expression of Aggrecan, Collagen I and II was quantified by real time PCR.

The physico-chemical study showed the impact of the HA-Tyr content on the hydrogel physical properties. At low HA-Tyr content (less than 0.4 %), the composite behavior was driven by collagen. Hydrogels exhibited a fibrillary network and were characterized by low mechanical properties. From 0.8% HA-Tyr, the mechanical properties and the hydration degree increased to reach those of NP (5kPa) when 2% HA-Tyr was added. Below 0.4% HA-Tyr, encapsulated cells contracted hydrogels after one week in culture. From 0.8%, hydrogels, MSCs did not contract hydrogels and their mechanical properties were stable over the time course of the experiment. With a high HA-Tyr content, cells did not proliferate, suggesting their commitment towards differentiation. At low content, MSCs spread and adopt a fibroblast like morphology. On the opposite, cells encapsulated within hydrogels at high HA-Tyr content were more rounded and resemble NP cells. The gene expression quantification showed that MSCs orientated towards a NP cell phenotype. When 2% HA-Tyr was used, cells highly expressed NP cells markers, i.e Aggrecan and Collagen II, and weakly expressed Collagen I. In contrast, cells encapsulated in hydrogels with a low HA-Tyr content weakly expressed these NP cell markers.

Taken together, these results show that Collagen/Hyaluronic Acid Composite Hydrogels with a high HA content (2%) mimic the physical properties of the Nucleus Pulposus and promote

the differentiation of MSCs into NP cells. Hence, these hydrogels could be useful for IVD regeneration.

keywords: collagen, hyaluronic acid, intervertebral disc degeneration, composite hydrogels, mesenchymal stem cells.

83767255989

PROTEOMIC CHARACTERISATION OF FOETAL NOTOCHORDAL CELLS TO INFORM INTERVERTEBRAL DISC DEVELOPMENT AND STEM CELL DIFFERENTIATION

Lizzy Ward (University of Manchester, Manchester, United Kingdom), Richard Unwin (University of Manchester, Manchester, United Kingdom), Andrew Dowsey (University of Bristol, Bristol, United Kingdom), Judith Hoyland (University of Manchester, Manchester, United Kingdom), Stephen Richardson (University of Manchester, Manchester, United Kingdom)

Loss of large, vacuolated notochordal cells (NCs) from the human intervertebral disc (IVD) is thought to initiate degeneration and associated back pain. It is therefore hypothesised that implantation of NCs may halt or reverse degeneration and thus relieve back pain. However, NCs are lost in early childhood, therefore iPSCs differentiation to NCs offers a clinically-viable cell source. Here we aimed to characterise the proteomic profile of the human foetal NCs, versus surrounding annulus fibrosus (AF) cells. FACS sorting for CD24, a known NC marker, was used to isolate NC (CD24+) and AF (CD24-) cells from microdissected human IVDs (14-15 weeks post-conception, n=3). Following iTRAQ isobaric tagging, TripleTOF mass spectrometry and subsequent bioinformatics, differential protein expression was validated by immunofluorescence. Our study revealed 100 up-regulated and 8 down-regulated proteins in the CD24+ population ($-1.5 \geq FC \geq 1.5$, $P \leq 0.1$), including known (e.g. keratins 8 and 19) and novel phenotypic markers. Ingenuity Pathway Analysis (IPA) revealed pathways known to play an important role in NCs and IVD homeostasis, such as NRF2-mediated oxidative stress response and caveolar-mediated endocytosis, and putative upstream regulators known to be active in NCs e.g. TGFβ1 and SMAD3. The same analysis predicted many previously unknown proteins, pathways and regulators to be active or repressed in NCs. Together, these results validate our method as a powerful tool for isolation and proteomic analysis of foetal NC cells and reveal novel proteins and pathways of potential use in the development of future strategies for the study and treatment of IVD degeneration, including in the development of protocols to direct differentiation of pluripotent stem cells towards NCs.

keywords: intervertebral disc, proteomics, stem cell

S68
Human brain organoids versus assembloids approach for neurodevelopmental studies
Room: S4 A
(28 Jun 2022, 15:30 - 17:00)

Conveners: Chiara Rinoldi, Leonora Bużańska, Arti Ahluwalia

62903406129

DEVELOPMENT OF THE INTEGRATED HUMAN BRAIN ORGANOIDS

In-Hyun Park (Yale University, New Haven, United States)

Brain organoids represent the 3D tissues that recapitulate the structure and function of the developing human brain. Much efforts have been made to advance the regionalization and to utilize the brain organoids to study human diseases. We developed region-specific cortical organoids and used them to study Rett syndrome. Since neuroectoderm differentiation of the human embryonic stem cells (hESCs) is an essential first step in brain organoid formation, the majority of cells in brain organoids are of the neuroectoderm origin. However, the cells that comprise the blood vessel in brain, and the residential immune cells in brain are from the mesoderm. In order to implement the mesoderm cells in brain organoids, we genetically engineered the hESCs to express the transcription factors that facilitate the formation of vascular-like structure and microglia-like cells. The vascular-like structures highly improved the quality of the brain organoids, dramatically decreasing the cell death, and increasing the neural maturation. The microglia-like cells demonstrated the innate immune function such as phagocytosis. Overall, our engineered brain organoids provide the essential mesoderm-derived cells in neuroectoderm-oriented brain organoids that play critical roles in brain function.

keywords: Brain organoids, Stem Cells, microglia

62903404146

IN VITRO MODELING OF HUMAN BRAIN REGION INTERACTIONS

Daniel Reumann (Institute of Molecular Biotechnology Austria (IMBA), Vienna, Austria), Jürgen Knoblich (Institute of Molecular Biotechnology Austria (IMBA), Vienna, Austria)

The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We use cerebral organoids to recapitulate those features in vitro and to test their role in human disease.

While the human brain is subdivided into distinct functional areas along its rostro-caudal and dorso-ventral polarity axes, brain organoids are currently limited by the lack of such polarity. To overcome those problems, we have used two approaches. First, we have introduced localized engineered sources of morphogens that can induce polarity in elongated organoids. Second, we have fused organoids resembling distinct brain areas to recreate polarity axes. We will present approaches to recreate functionally integrated multi-part organoid systems and their application to model neuro-developmental and neurodegenerative brain disorders.

keywords: brain organoids, assembloids, neurodevelopment, neuropathologies

31412737644

ADVANCED IN SILICO METHODS FOR ORGANOID AND ASSEMBLOID DESIGN

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Introduction
In silico methods integrate physical and biochemical models with computational tools, and are a powerful support for tissue engineering. They are particularly relevant for studying organoids and assembloids: the multiplicity of parameters which condition organoid growth and morphology can be explored in virtual models, facilitating experimental design, and enabling prediction and extrapolation of behaviour and function. Here we use statistical physics and evolutionary algorithms to predict how cells in cerebral constructs cooperate to share resources (oxygen) and generate functional forms.

Methodology
Kleiber's Law (KL) is a universal law of biology, stating that the metabolic rate of an organism scales with its body size according to a quarter-power law 1. Its pertinence for designing human-relevant in vitro models 2–4 has been highlighted. However, KL is formulated as a deterministic framework, although fluctuations and heterogeneity are inevitable, and known to shape the response of biological systems to external perturbations (nanoparticles, viruses). We generated joint distributions of construct masses and metabolic rates, developing new statistical tools to test whether and in which organoid size range a generalized stochastic formulation for KL 5 applies. KL is combined with physical, metabolic and mechanical constraints to generate in silico models of organoids with different shapes and sizes to identify optimal design criteria for functional models.

Results
We found that stochasticity significantly restricted the range of construct sizes complying with KL, implying that to date many cellular models may lack translatability 6. Evolutionary algorithms based on the optimization of a cost function which incorporates resource uptake, surface energy and cooperative metabolic effort have enabled the generation of model datasets. These studies are used to assess to what extent morphometry or metabolic phenomena affect organoid formation and growth and to identify experimental design specifications to obtain constructs with translational value.

Conclusions
In silico models enable the definition of criteria for designing brain organoids and assembloids with translational value and hence useful for robust in vitro to in vivo extrapolation, paving the way towards predictive and precision medicine and reducing animal tests. Ongoing experimental validation suggests that three dimensional constructs manifest cooperative behaviour and that rather than being discarded, variability and fluctuations in organoids confer robustness.

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keywords: brain organoids, assembloids, computational modelling, oxygen, allometric scaling

62825466227

PHYSIOLOGICAL NORMOXIA INFLUENCE NEURAL CELL FATE THROUGH CHANGES OF MITOCHONDRIAL DYNAMICS AND GLYCOLYSIS/OXPHOS SWITCH IN HUMAN BRAIN ORGANOID MODEL

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Introduction
Mitochondrial dynamics and metabolic alterations play a pivotal role in neuron maintenance and differentiation during early human neurodevelopment. Brain organoids (BO) derived from human induced pluripotent stem cells (hiPSC) provide a unique model to study developmental stage-specific sensitivity of mitochondrial dynamics to different microenvironmental cues. Our previous experiments performed on hiPSC-derived neuronal cultures showed that physiological normoxia (5% O2) impacts neural to glial cell fate by increasing expression of the astrocytic markers and lowering expression of the neuronal markers. In this work, we used a brain organoid model from hiPSCs grown in two different oxygen conditions – 5 and 21% O2 - to decipher the influence of mitochondrial dynamics on neural cell fate.

Methods
BO were generated from hiPSCs and cultured either in 21% (control) or 5% O2 (physiological normoxia). Then, 11-day neurospheres (11D-N), 44-days (44D-BO) and 4-month brain organoids (4M-BO) were collected. BO metabolism was evaluated by monitoring the ATP levels and through Alamar Blue assay. Changes in expression for markers specific to neural stem cells (Nestin, Pax6), neuronal cells (MAP2, bTubIII), glial cells (GFAP), proliferation (Ki-67), mitochondria (MAB1273) were determined by immunofluorescence labelling. To determine the effect of low oxygen on 44D-BO at the genomic level, RNA-seq experiment was performed.
To assess changes in mitochondrial networks, organoid sections were immunostained with anti-MAB1273, a mitochondrial surface protein. Confocal and structured illumination microscopy images were acquired on a Zeiss LSM 780. Image processing and quantitative analysis of the mitochondrial morphology were determined using ZEN software, FIJI plugins (Mitochondrial Analyzer plugin, MorpholibJ plugins and 3D Manager) and ad hoc Matlab routines.

Results
Our mitochondria analysis framework revealed changes in mitochondrial network parameters throughout brain organoid development and show that physiological normoxia affects key parameters of mitochondrial morphology in a developmental stage-specific manner. The most noticeable effect of low oxygen conditions on mitochondrial shape, connectivity and size predictors was observed at the stage of 44-day brain organoids therefore most of the analyses were performed at this stage. Metabolic assays showed a lower rate of metabolism, which is accompanied by a noticeably smaller diameter of 44D-BO in 5% O2 compared to 21% O2 controls. Furthermore, 3D analysis of segmented mitochondrial objects from the super-resolution microscopy images revealed significant alterations in mitochondrial volume, surface area, equivalent diameter, sphericity in the cortical zone of BO grown in 5% O2 with respect to 21 % O2 -cultured ones. Transcriptomic analysis revealed upregulation of genes involved in:

HIF-1 signaling pathway, glycolysis/gluconeogenesis, central carbon metabolism and pyruvate metabolism in 44D-BO grown in 5% O2. Our results suggest that in physiological normoxia, glycolysis prevails over oxidative phosphorylation (Oxphos). This is accompanied with decreased expression of neuronal markers (βtubIII, MAP2) and increased level of glial marker (GFAP) in brain organoids grown in low oxygen conditions compared to controls, confirming the influence of low oxygen on neural to glial cell fate transition.

In summary, this study shows that oxygen conditions influence neural fate by inducing changes in glycolysis/Oxphos ratio and mitochondrial dynamics in a stage-specific manner during brain organoid development.

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keywords: cerebral organoid, brain development, oxygen conditions, mitochondrial dynamics, neural cell fate

31451707929

ESTABLISHING TOOLS TO STUDY THE EMERGENCE OF CELLULAR DIVERSITY IN THE HUMAN BRAIN

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In this seminar, I will provide a basic overview of the developmental mechanisms underlying the emergence of functional areas in the cerebral cortex. I will start by providing an overview of studies performed in rodents. Subsequently, I will transition to describe our studies in human, highlighting similarities and differences in progenitor cell diversity between mice and humans, I will subsequently describe how single cell transcriptomics and single cell epigenomics have helped us to identify modules of co-regulated and co-functional genes during human cortical development. Finally, I will describe our latest work on deciphering the role of developmental signaling pathways in promoting cortical arealization in the human brain.

keywords: brain development

Poster Sessions

PS01
3D in vitro tissue-engineered cancer/disease models – Session I

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3D ADIPOSE-LIKE TISSUE ANALOGUES BEARING INFLAMED AND HYPERTROPHIED ADIPOCYTES TO STUDY OBESITY IN VITRO

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INTRODUCTION: The increased prevalence of obesity over the last few years has intensified the need for physiologically relevant models for the characterization of the mechanisms regulating obesity-linked adipogenic and inflammatory responses. In obesity, there is an unhealthy expansion of adipose tissue, at the expense of hypertrophic processes responsible for the enlargement of adipocytes. This leads to the generation of a chronic low-grade inflammation, altered adipokine secretion and increased lipolysis. Current in vitro obesity research focuses mainly on adipose tissue models on two-dimensional cell culture that fall short of mimicking the three-dimensional (3D) in vivo environment. To surpass that, this work aims at developing 3D adipose-like tissue analogues bearing hypertrophied adipocytes within an inflammatory milieu.

METHODS: Human adipose-derived stem cells were seeded at sub-confluency and cultured for 3 days with ascorbic acid supplementation and then for 6 days in adipogenic differentiation medium plus 9 days in adipogenic maintenance medium1. The generated cell sheets were then submitted to different time combinations of TNF-α (100 ng/mL), an inflammatory cytokine, and palmitic acid (PA) (0.5 mM), a saturated fatty acid that promotes adipocyte enlargement, for further 13 days. Adipocyte size (image analysis), lipid accumulation (LipidTOX) and glycerol release (Glycerol Assay Kit) were assessed as adipogenic indicators, and the secretion of TNF-α and IL-6 (ELISA) was used as an indicator of the inflammatory status. The expression of adipogenic (PPARγ and FABP4) and lipolytic (pHSL, ATGL and PLIN) markers was determined at the mRNA (RT-PCR) and protein (western blot) levels. Additionally, to further increase the complexity of our system, differentiated cell sheets were stacked together to produce 2- and 3-layered constructs, which were then submitted to the same treatments as the single cell sheets and assessed regarding the same parameters.

RESULTS & DISCUSSION: Altogether, our analysis showed that the combination of hypertrophic and inflammatory stimuli (PA+TNF-α) for 13 days, and the treatment with PA alone for 10 days followed by a combination of PA+TNF-α for 3 days resulted in adipogenic cell sheets with higher TNF-α and IL-6 secretion, larger adipocytes with increased accumulation of neutral lipids and increased glycerol release. These features were found in both single cell sheets and 3D constructs.

CONCLUSION(S): These results showed that by tuning the culture conditions of cell sheets with inflammatory and hypertrophic stimuli we are able to modulate their adipogenic phenotype.

Overall this allowed obtaining 3D adipogenic analogues presenting enlarged adipocytes with a pro-inflammatory profile resembling the characteristics of the obese adipose tissue.

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keywords: obesity, inflammation, adipocyte hypertrophy, cell sheet engineering, 3D models

52354552266

3D PROSTATE CANCER IN VITRO MODELS

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Prostate cancer is one of the most commonly diagnosed cancer in men, and the 5th leading cause of death worldwide1. The lack of the of robust and biologically relevant in vitro model systems that precisely recapitulate the pathophysiology of the human disease delay our understanding of the disease, and thus the discovery of the treatments. Owing the fact that tumor microenvironment plays an essential role in tumor progression and metastasis, functional materials mimicking the prostate extracellular matrix (ECM) properties and including stromal components will provide enhanced models 2. Bioprinting has emerged as a promising technique to fabricate 3D in vitro models with precise control of constructs' architecture and cell spatial distribution3. This work describes the design and fabrication of prostate cancer 3D in vitro models using the combination of both advanced biomaterials and bioprinting. With this strategy we aim to better mimic properties of the native prostate tumour microenvironment, offering new models to study how prostate cancer progresses in vitro. Successful bioprinting methods rely on the selection of a suitable bioink that enables control of 1) the resolution of the printing process and 2) deposition of known bioactive materials and viable mammalian cells. Alginate-based bioinks were selected due to the biocompatibility, ease of functionalization, inherent shear thinning property, printability, and quick gelation with divalent ions (i.e.Ca^{2+}). Alginate maintain stable 3D printed tumor models, with reduced degradation in vitro thanks to the absence of alginate degrading enzymes. To improve cell adhesion, oxidized alginate (OA), with target 50% oxidation degree, to control the amount of aldehyde groups able to covalently link active peptides. As laminin is one of the main proteins of prostate ECM and the main constituent of basement membrane, we have selected IKVAV and AG73, known to promote cell adhesion and mimic laminin, and conjugated with OA (i.e. OA-PEG-pep).
Furthermore, alginate allows control over mechanical properties of formed hydrogels and in the typical range of prostate tissue (~ 1-10 kPa). Prostate cancer cells (PC-3) were encapsulated at a concentration of 1 × 106 cells/mL in alginate-based hydrogels (e.g. 1% (w/v) OA-PEG-pep / 1% (w/v) alginate/ 3% (w/v) gelatin), with final PEG-Pep concentration per gel formulation of 0, 100, and 400 µM. Hydrogels were physically crosslinked with $CaCl_2$ (0.1 M or 0.3M) for 10 min at 37°C. PC-3 cells adaption to the microenvironment, e.g. physico-chemical and mechanical properties, was evaluated by assessing cellular viability, proliferation, morphology, and expression of epithelial to mesenchymal transition markers. Finally, extrusion-based printing technology was used to print spatially defined system with spatial control over stiffness and cellular deposition of PC-3 with cellular viability (75-85%). Future works will involve the co-culture of PC-3 with cancer associated fibroblasts to evaluate the contribution of stromal cells. Bioprinted models measured approximately (2 mm x 2 mm x 1 mm), where cancer cells (PC-3) constrained to the central core of the printed constructs. Designed alginate based bioinks enables printing 3D prostate constructs with high cell viability providing by that biomimetic tissue to study prostate cancer progression and metastasis.

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keywords: Prostate Cancer, 3D in vitro models, Bioprinting

94238105106

A TAILORED BIOREACTOR SYSTEM COMBINED WITH A BIOPRINTED MICROVESSEL SUBSTITUTE ENABLES THE INVESTIGATION OF CHEMOTAXIS IN IN VITRO MODELS

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The combination of 3D bioprinting and tailored bioreactor designs allows the fabrication of advanced in 3D in vitro models. However, there is a lack of biomimic vessel models focusing on chemotaxis, which enable the investigation of the effect of simultaneous external stimuli. In this study, we propose a novel bioreactor system combined with bioprinted microvessels embedded in fibrin-based extracellular matrix substitutes (ECM) for that purpose. The long-term stability of fibrin-collagen and fibrin-gelatin blends was investigated using an in vitro degradation test. Hydrogel samples were incubated in PBS and the weight was measured for up to 28 days. The permeability of fibrin-collagen and fibrin-gelatin blends were investigated using a tailored transwell assay. The transwell membranes were covered with hydrogel blends and the diffusion of fluorescein isothiocyanate labelled albumin from bovine serum (FITC-BSA) from the upper well into the PBS-filled lower well was measured using a microplate reader. The release of growth factors typical for inflammation (tumor necrosis factor α (TNF-α), stromal derived factor 1 (SDF-1)) from the hydrogel blends was investigated using an ELISA-kit. Microvessels mimicking arteries were fabricated using coaxial bioprinting technique by combining an endothelial cell-laden (HUVEC) sacrificial gelatin core with a smooth muscle cell-laden (SMC) fibrin-based shell. The channels were embedded into a fibrin-based ECM cultivated using a perfusion pump and a tailored, 3D printed bioreactor system for up to 28 days. The middle part of the bioreactor incorporated the final hydrogel construct of 3 mm thickness. Two exterior reservoirs were separated from the hydrogel construct by permeable membranes. Medium containing cytokines and chemical stimuli were injected into the exterior reservoirs and diffuse into the hydrogel construct and the perfusable microvessel substitute. The cellular organization of HUVECs and SMCs was investigated using immunostaining and confocal and two-photon microscopy. All hydrogel blends showed hydrolytic stability for at least 21 days. Swelling and shrinking of fibrin-gelatin blends was tuned by heat pretreatment of the gelatin component. Fibrin-collagen blends initially shrunk while the shrinking was reduced by increasing thrombin concentration and control of pH and temperature. Fibrin-gelatin blends provided twice the permeability of fibrin-collagen blends within the first 10 h. However, both blends levelled at a similar maximum permeability after 48 h. Gels with higher polymer concentration and hence denser microstructure showed lower permeability within the first hours compared to lower concentrated gels. The release of cytokines was distinctly higher from fibrin-gelatin blends (10-20 ng/ml for TNF-α; ~10 ng/ml for SDF-1) compared to fibrin-collagen blends (~5 ng/ml for TNF-α; ~3 ng/ml for SDF-1) after 24 h. A functional HUVEC monolayer lined the inner lumen of perfusable channels of approx. 500 µm in diameter. SMC showed high viability (<80%) and characteristic stretching inside the gels. In conclusion, we present a novel tailored bioreactor system which can be used to investigate the effect of external chemoattractants on angiogenesis, chemotaxis, intravasation, or extravasation. In combination with bioprinted microvessel substitutes, it represents a versatile and easy-to-use approach and can be used for a broad variety of tissue engineering applications.

keywords: Biofabrication, bioreactors, in vitro models, bioprinting, chemotaxis

62825468677

A THREE-DIMENSIONAL DYNAMIC MODEL OF OVARIAN CANCER BY USING A PERFUSION BIOREACTOR

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Introduction:
Ovarian Cancer (OC) research is limited by the lack of an appropriate in vitro model of the tumor microenvironment. We utilized a perfusion bioreactor, suitable for the culture of up to 40 cell-seeded scaffolds (1), at velocities mimicking the cellular conditions in the extracellular fluid flow (2), to construct a new OC model. Our hypothesis was that operating the reactor at low volumetric velocities, with a vertical flow model will allow better homogenous cultivation, compared to a horizontal fluid, highly affected by gravitation forces.

Methods:
Velocities and shear stresses throughout the reactor body were simulated by ANSYS Fluent, at different velocities, of vertical or horizontal flow. 433 and ES2 OC cells were seeded into alginate macroporous scaffolds and cultured in the perfusion bioreactor for 3 days under the simulated conditions. Cell viability was examined by Presto Blue assay. RT-PCR analysis was conducted for Sphingosine-1-Phosphate receptors (S1PRs), associated with OC. The mRNA expression levels were compared to other culture methods (monolayer, static seeded scaffolds, spheroids) and to OC samples of primary tumor and effusions.

Results:
ANSYS Fluent simulation indicated higher homogeneity at 50mL/h vertical flow, compared to horizontal flow conditions. ES-2 seeded scaffolds culture at 50 mL/h vertical flow, resulted in more homogenous cellular viability, compared to horizontal flow; supporting the simulation results. mRNA expression levels of S1PR1 and S1PR2 were shown to be significantly lower when 500 mL/h volumetric velocities were applied, compared to 50 mL/h and static conditions ($p<0.05$); emphasizing the necessity of specific velocity for the OC in vitro model. 433-seeded scaffolds, cultured at 50 mL/h resulted in S1P receptor mRNA expression levels, similar to those of the primary OC samples, compared to monolayer, static scaffold, and spheroid cultures.

DISCUSSION & CONCLUSIONS:
Vertical perfusion flow at relatively low velocity was found to be efficient for constructing novel in vitro models of OC Primary and effusion.

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keywords: Ovarian Cancer, model, dynamic, perfusion, bioreactor

62825447946

A TISSUE ENGINEERING APPROACH TO STUDY BONE METASTASES IN VIVO

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Introduction
Bone metastases are the most common cause of cancer-related pain and often lead to complication such as bone fractures and spinal cord injury, all of which can severely erode patient's quality of life. Even tumors such as breast cancer that can be now early diagnosed, leading to a positive resolution, may present increased morbidity and mortality if they metastasize to bone. The development of models allowing the study of cause mechanisms and new therapies represent a great challenge in cancer research. Animal models are essential for the study and characterization of these mechanisms. The ideal model should be clinically relevant and reproducible and should recapitulate human pathology. Different models are already available, each one with its own advantages and disadvantages. However a unique and standardized model has not been defined yet.

Methods
Our aim was to use a tissue engineering approach to create a mouse model, which allows to obtain a suitable environment for bone metastasis, starting from human cancer cells deriving from breast cancer. Human mesenchymal stromal cells (hMSCs) were seeded on a biocompatible and porous scaffold based on hydroxyapatite and β-tricalcium phosphate (MBCP+). The constructs were implanted subcutaneously in nude mice, to induce human bone growth. Once human ossicle was obtained in this "foreign" environment, human breast cancer cells were inoculated directly on the implant site, or intravenously through the tail. We injected luciferase-expressing breast cancer cells and the colonization and metastases formation were followed by IVIS. After two weeks mice were sacrificed, and the explants analysed by histology or processed to recover the colonizing cells. Selection of tumor cells was performed thanks to the expression of resistance gene for zeocine antibiotic. Culturing explants in selection medium (in presence of zeocine), only tumor cells which colonized the tissue will be able to attach and grow.

Results
MDA-MB-231 metastatic breast cancer cells or MCF-7 cells were injected intravenously in mice previously implanted with MBCP+± hMSCs to obtain human bone. At first, MDA-MB-231 colonized the lungs, but they were able to reach the human bone within two weeks, and colonizing cells can be easily harvested from the explants. No colonization was observed in mouse skeleton. Interestingly, in mice implanted with MBCP+ alone (empty control), cancer cells did not reach the scaffold, suggesting a species-specific mechanism. MCF-7 cells were not able to metastasize, and no cells were recovered after explants.
Discussion and conclusions:
This system allows to study the mechanism by which breast cancer metastasizes to the bone, creating a species-specific environment. It could be an excellent tool for testing new combinations of drugs and, by using the patient's cells directly, it could become a tool for the development of personalized therapies. Further investigations are required to characterize the metastatic cells on molecular level to shed light on the mechanism of action.

keywords: tissue engineering, breast cancer, bone metastases

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ALGINATE MICROFIBERS WITH IMMOBILIZED CANCER CELLS AS A 3D CANCER MODEL FOR ANTICANCER DRUG TESTING

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Introduction
The development of reliable preclinical systems for anticancer drug testing has become an imperative considering that over 96% of oncology drugs that reach clinical trials ultimately fail [1]. Traditional cancer models comprising monolayer cell cultures (2D) where the cells adhere to the flat surface of a culture flask cannot recapitulate the complex 3D structure of in vivo cancers. To overcome this problem, significant effort has been put into the development of more adequate biomimetic 3D model systems for anticancer drug testing. In this study we demonstrate the potential of alginate microfibers with immobilized cancer cells as a 3D cancer model for anticancer drug research.

Methodology
Immobilization of cancer cells (NCI-H460 cell line) in alginate microfibers was achieved by simple extrusion. A mixture of Na-alginate solution and cell suspension (4×106 cells/ml, 2.8 % Na-alginate) was manually extruded through a blunt edge stainless steel needle (25G) into the gelling solution containing 0.18 M Ca2+. Formed alginate microfibers with immobilized cells were then cultured for 48 h in the culture medium after which period the cells were treated with the anticancer drug cisplatin at concentrations of 1, 5, 10, 25 and 50 μM. Untreated cells immobilized in alginate microfibers served as a control. Simultaneously, the same cisplatin concentrations were applied to the cells in 2D monolayer cultures, with untreated cells as a control. After another 48 h, cell viability was assessed by the MTT assay based on the reduction of the MTT dye into formazan in metabolically active cells. After the addition of the dye (0.5 mg/ml MTT), cells in both 3D and 2D cultures were incubated for 4 h at 37°C, followed by dissolution of the formed formazan crystals with dimethyl sulfoxide and measurement of the absorbance of the obtained solutions at 570 nm. The cells in microfibers were also examined regarding morphology by optical and fluorescence microscopy.

Results
Cancer cells were successfully immobilized in alginate microfibers retaining their normal morphology and viability, confirmed by optical microscopy and MTT staining. After the treatment with anticancer drug different cell viability results were obtained in 3D cultures compared to 2D cultures. In 2D cultures, the half-maximal inhibitory concentration (IC50) of cisplatin was shown to be 5 μM, while in 3D cultures the concentration of 5 μM reduced the cell viability by only 24 %. Furthermore, the IC50 value in the 3D culture was not achieved in the applied range of concentrations, with a reduction in cell viability of 43 % at the highest applied cisplatin concentration, 50 μM. In comparison, the cisplatin concentration of 50 μM in 2D cultures reduced the cell viability by 76 %.

Conclusion
The preliminary study of anticancer drug testing in the 3D cancer model comprising cancer cells immobilized in alginate microfibers showed increased resistance of the cells to the anticancer drug cisplatin compared to cells in 2D cultures. This result indicates that such 3D systems could be used as more reliable cancer models in anticancer drug research.

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keywords: 3D cancer model, alginate hydrogel, human non-small cell lung carcinoma, cisplatin, tumor engineering

83767266159

BIOENGINEERING THE HUMAN BONE NICHE WITH HIGH ADIPOSE CONTENT TO STUDY ADVANCED CANCER IN VITRO AND IN VIVO

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Introduction
In advanced prostate cancer (PCa), cancer cells preferentially metastasise to bone. At this stage, survival rates drop from 56% to 3%1 and most treatment options for patients remain palliative. The bone metastatic niche (BMN) is indeed a complex microenvironment with various cell populations that have different roles in cancer cell migration, survival, and therapy resistance2. While osteoblasts and osteoclasts fuel metastastic establishment and progression in the bone microenvironemnt, bone marrow adipocytes (BMAs) account for 70% of the marrow and have been identified as a potential contributor3. To date, there is however a lack of relevant models to study the complex relationship between PCa cells and adipocytes within the bone microenvironment, as most models either employ murine cell lines, high fat diet mice4, and white adipocytes instead of bone marrow adipocytes, impairing to truly dissociate the role of human BMAs in PCa progression in bone. Therefore, we have developed sophisticated bioengineered humanised models with a high human adipose content that better replicate the complexity of the BMN, in vitro and in vivo.

Methodology
We developed a humanised fatty BMN by co-culturing primary human osteoblasts, human bone-marrow derived mesenchymal stem cells differentiated into adipocytes and androgen-sensitive human PCa cells (LNCaP and C4-2B), encapsulated into Gelatin Methacryloyl (GelMA) hydrogels5 for in vitro and in vivo analysis. Construct characterization was done by evaluating cell viability, proliferation and differentiation, spheroid formation and osteoblast mineralization. After four weeks of osteogenic differentiation and mineralization in vitro, the bioengineered mineralized tissues were subcutaneously implanted into male NSG mice. Six weeks later, adipose/cancer constructs were implanted in the same subcutaneous pocket to create a fatty BMN, as to assess the effects of human adipocytes on cancer progression. In vivo bone formation was followed by computed tomography (CT) and cancer progression by bioluminescence imaging. After five weeks of co-culture, the humanised niche was characterized ex vivo by microCT and histology.

Results
Construct optimization allowed to culture each cell type independently or concomitantly in GelMA hydrogels for up to six weeks in vitro, resulting in appropriate adipogenic differentiation, cancer spheroids formation and osteoblast mineralization. In vivo data showed bone formation with high mineralization overtime and the morphology of human native bone, including a cortical shell and trabecular centre. In vitro and in vivo, cancer progression was observed for the most aggressive cancer cell line (C4-2B) and was enhanced by the presence of human adipocytes.

Conclusions
The bioengineered models presented here offer an advanced platform for bone metastatic cancer research, such as the study of the effects of conventional therapies as well as for the screening of novel therapies on the bone tumour microenvironment, which is the next step of this study.

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As a past co-chair of TERMIS-AP 2019 and part of the executive committee, I would be interested to co-chair the corresponding symposium, 3D in vitro tissue-engineered cancer/disease models – Session I.

keywords: Bone marrow, hydrogels, prostate cancer, adipocytes

52354517466

BIOMIMETIC THREE-DIMENSIONAL IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER UTILIZING GELMA HYDROGELS

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Introduction: The burden of central nervous system (CNS) diseases continues to increase especially with an aging global population. The interface between the brain and the blood is governed by the specialized endothelium which composes the blood-brain barrier (BBB). Brain metastasis has a high rate of incidence from melanoma, breast and lung cancers and the BBB is strongly involved in these events. The breakdown or malfunction of the BBB plays a pivotal role in CNS diseases. Moreover, drug delivery to the brain is often hampered by the tight BBB. This study aims to develop a biomimetic BBB model that closely mimics the BBB physiology to investigate drug delivery and cancer metastasis to the brain.
Methods: The BBB model was created by co-culturing human aortic endothelial cells on top of human astrocytes encapsulated in gelatin-methacrylate (GelMA) hydrogels. The astrocytes were encapsulated in GelMA hydrogels of different concentrations (5%, 7.5% and 10% w/V) to study their growth and proliferation in a 3D environment. The viability of astrocytes was assessed via live/dead assay. The morphology of the astrocytes was evaluated by fluorescent imaging. The endothelial cells were cultured on top of the hydrogels on inserts having 8 μm pores where endothelial cells cultured on inserts with 0.4 μm pores with astrocytes cultured on the bottom side were used as controls. The barrier function was studied by transendothelial electrical resistance (TEER) and Evan's blue albumin (EBA) permeability assay. Tight junction protein, ZO-1 formation of the endothelial cells was assessed by immunofluorescent staining.
Results: Astrocytes encapsulated in 5%, 7.5% and 10% GelMA hydrogels exhibited a high viability (>80%) with no statistical significance between the different concentrations. Encapsulated astrocytes had a star-shaped morphology in the hydrogels. TEER measurements of endothelial cells cultured on GelMA hydrogels encapsulated with or without astrocytes showed higher values when compared to the plastic counterparts. Despite higher TEER values, the permeability of EBA were higher in the hydrogel models. This is due to the different pore size of the inserts used in the hydrogel models which only allowed ~3% of EBA to cross when compared to an empty insert while the plastic counterparts allowed ~ 15%. The ZO-1 staining showed more tight junction formation in the GelMA hydrogel models with or without astrocytes.
Conclusion: This study utilizes biocompatible GelMA hydrogels which provide a 3D environment for the growth of astrocytes. The hydrogel model provides insight into the effect of extracellular matrix on cell growth and function. The hydrogel model will allow the studies of cancer invasion and migration required to investigate the interaction of the BBB with metastatic cancer cells. The 3D biomimetic BBB model provides a tool to investigate brain metastasis and the administration of drugs to the brain with high fidelity.

keywords: blood-brain barrier, 3D culture, GelMA hydrogel, tissue engineering, drug delivery

31412727804

COAXIAL PRINTING OF CONVOLUTED PROXIMAL TUBULE FOR KIDNEY DISEASE MODELLING

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Introduction
Despite increased global incidence of kidney disease, their mechanisms are yet not fully understood. Among the different segments of the nephron, the functional unit of kidneys, the proximal tubule (PT) is most susceptible for toxicant-induced injury because of its role in xenobiotic secretion and reabsorption. Moreover, genetic defects in transporters may lead to metabolic complications and tubulopathies. To gain insight in these diseases, it is of paramount importance to develop representative biomimetic in vitro models. The often-applied 2D cell models are based on the use of PT epithelial cells (PTECs) cultures. 3D (bio)printing offers new modeling alternatives to incorporate cell-extracellular matrix (ECM) interactions as it has been repeatedly shown that both ECM curvature and composition are fundamental for the adequate behavior of PTECs. Here, we applied co-axial printing to create a convoluted channel within a gelatin-based microfiber to model the convoluted structure of the PT and address the ECM-cells interaction in a diseased model. For that, we included a cystinosin-deficient (CTNS-/-) cell line to model cystinosis, a currently uncurable kidney tubulopathy.

Methodology
A 3D printing system consisting of syringe pumps, heaters, coaxial needles, and a silicon holder was designed. A gelatin/alginate-based ink was formulated to allow printability while maintaining structural properties. Fine-tuning of the composition, printing temperature and feeding rate allowed an optimal biomaterial ink viscosity. Calcium chloride and microbial transglutaminase were used to stabilize the biomaterial ink. To study the stability of the hydrogel, a degradation assay was conducted. Healthy conditionally immortalized PTECs (ciPTEC), and CTNS-/- cells were seeded to mimic two genotypes of PT. Immunofluorescent stainings for cytoskeleton organization (F-actin), polarization markers (a-tubulin, Na+K+-ATPase), ECM-production (collagen IV), and barrier-formation (inulin-FITC leakage) were performed to evaluate the performance of the engineered PT.

Results
The printed microfibers exhibited prolonged structural stability (42 days) and cytocompatibility in culture. Healthy and cystinotic cells showed homogenous cytoskeleton organization upon 14 days of culture in the helical microfibers, as indicated by staining for filamentous actin, measuring barrier-formation and assessing polarization with the apical marker a-tubulin and the basolateral marker Na+K+-ATPase. Cell viability was slightly impaired in cystinotic cells upon prolonged culturing for 14 days. Finally, CTNS-/- cells showed reduced apical transport activity by two efflux pumps, viz. breast cancer resistance protein (BCRP) and multidrug resistance-

associated protein 4 (MRP4).

Conclusion(s)
Our novel printing device showed potential to mimic a 3D environment compatible with healthy PT and tubulopathy modeling. By further improving this setup, new insights in kidney disease development and progression can be gained. This eventually aids in new treatment options.

keywords: coaxial 3D printing, biomaterials, proximal tubule, kidney, in vitro modeling

83767222655

DERIVED TUMOR EXTRACELLULAR MATRIX IS SUITABLE TO 3-DIMENSIONAL CELL GROWTH AND PROVIDE A PROPER ENVIRONMENT FOR TUMOR CELLS

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Introduction: The extracellular matrix (ECM) is a highly organize structure that represents the major structural component of the tumor microenvironment. The ECM is a non-cellular network of proteins that provide to the tumor cells the capability to invade, proliferate and migrate. Indeed, the tumor microenvironment represents a dynamic scaffold, and the study of the ECM structure represents the first step to be overcome to understand cellular, molecular, and immunologic mechanisms of tumor response and resistance. A lot of well-known biomaterials were proposed to mimic the 3D tumor structure, however the cell adhesion to this structure did not represent the real tumor microenvironment due to the lack of the complex structure and interaction typical of the tumor.
To overcome these limitations, in this work was proposed to recreate a 3D tumor structure using the decellularized matrix of a native tumor as a scaffold that is colonized by different cell types allowing the study of the interactions between the tumor cells and immune system or the screening of the drugs for targeted therapeutic models.
Methodology: Murine B16f10 melanoma cells were expanded and injected subcutaneously to C57Black/6 mice to create a tumor. After two weeks we harvested the tumor and started the decellularization process (United States Patent 6743574B1.2004) using a cryotube placed in a tilling system (static system) and a dynamic system using a bioreactor (U-CUP). At the decellularization process follows the recolonization of tumor matrix by cells (GFP-MDA and NHI3T3). After 7 days the recellularized tumor were recovered and histological analysis was performed.

Results: The decellularization process removed all the cells from the tissue demonstrated by the absence of DAPI positivity in the slides and was not detrimental to the extracellular fibers, as confirmed by trichrome staining and collagen type VI. The decellularization process developed with both the methods was well.
To test the efficacy of the decellularized tissue as scaffolds for 3D culture, we executed different recellularization experiments. After 7 days of culture, in static system the cell attachment was limited just to the border of the tissue. In dynamic condition the cells appear more integrated into the decellularized scaffold. These differences were due to the collapse of the structure in static condition demonstrated by structural analysis of the decellularized tissue compared to the fresh one that showed an increase of ECM. This hypothesis was confirmed with SEM images, that showed a collapse of the matrix caused by fibres twisted on themselves, generating a complex net, impenetrable from cells.

Conclusions: Our result demonstrate that the murine melanoma decellularized tissue is suitable for 3D in vitro tumor microenvironment reproducibility when is treated in dynamic system that recreates conditions favorable to cell penetration into the decellularized matrix. So, this method could provide a new method to obtain a patient-personalized tumor, adequate for drug

screening that will eventually drive to the correct drug therapy for each patient.

keywords: tumor extracellular matrix, 3D models, decellularization, bioreactor, melanoma

62825426286

DESIGN OF A COMBINED COLLAGEN AND LUNG DECELLULARIZED EXTRACELLULAR MATRIX HYDROGEL FOR THE STUDY OF THE LUNG TUMOR MICROENVIRONMENT (TME)

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Introduction/background: Different hydrogels have recently been used to study the interactions between the different components of the tumor microenvironment (TME) in several types of cancer, including lung cancer. These studies try to emulate the characteristics of the tumor extracellular matrix. The pulmonary extracellular matrix is very complex. It varies in the different portions of the lung and includes variable amounts of collagen fibers of different diameters, elastic fibers, proteoglycans, and a wide variety of macromolecules. On the other hand, the tumor extracellular matrix has its own composition, different from that of the normal lung. Due to the difficulty in emulating the extracellular pulmonary matrix, strategies have been developed based on the use of decellularized tissue matrices that serve as a support for the culture of TME cellular components. Its application to the lung environment is difficult due to the due to the great variability in the composition of the extracellular matrix and the small size that most lung tumors tend to have. In this work we intend to generate a mixed collagen hydrogel that includes elements of the pulmonary extracellular matrix to use it as a structural support for the study the biology of cancer cells, non-tumor fibroblasts and cancer cells.

Methods: 4 mg/ml collagen hydrogels were generated. Rat lung tumor tissue was decellularized using Triton X-100. After histological validation of the decellularization protocol, the tissue was further digested with pepsin, lyophilized and a homogeneous powder was obtained by grinding a mortar and liquid nitrogen. The powder was combined with the collagen hydrogel. A549 cells were embedded in the manufactured hydrogel and cultured in DMEM culture medium supplemented with a 10% of fetal bovine serum, penicillin, streptomycin and fungizone for 72 hours in the presence or absence of TFGβ5 ng/ml. Morphological characteristic of A549 cells were studied by fluorescence staining of F-acting using phalloidin-rhodamine. Vimentin was studied by immunofluorescence. CDH1, CDH2 and VIM were analyzed by real time RT-PCR.

Results: The cells cultured in the hydrogel showed a stellate morphology, with abundant actin stress fibers. The expression of vimentin and the cell density were significantly higher in the cells cultured in dECM hydrogels compared to that cultured in collagen hydrogels. Effects of TGF beta in reference to the expression of EMT markers were more evident in the dECM hydrogels.

Conclusion: The manufactured hydrogel represents a suitable support for the culture of stromal cells isolated from lung cancer tissue. This prototype will serve as a basic support to generate complex culture systems that will allow us to study different physical factors (gel stiffness, mechanical-magnetic forces effects, etc.), biochemical factors or cellular elements conforming the TME.

keywords: lung cancer, organoids, tumor extracellular matrix, tumor microenvironment

31412721609

DEVELOPING A 3D MODEL OF COLORECTAL CANCER TUMOUR MICROENVIRONMENT

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Colorectal cancer (CRC) is the third most common cause of cancer-related deaths worldwide1. According to molecular signatures, it can be classified into four Consensus Molecular Subtypes (CMS) with CMS4 tumours being associated with worst relapse-free and overall survival2. CMS4 tumours are rich in mesenchymal stromal cells (MSCs) and have increased angiogenesis and inflamed immune phenotype. Understanding the cellular interactions in the tumour microenvironment (TME) will be essential for developing novel cell therapies for treating CRC such as CAR-T and CAR-NK therapies. Much of the research in the past has focused on 2D monolayer cultures or in vivo studies, however, successful clinical translation of therapeutics derived from these methods has been sub-optimal. 3D models offer a promising tool to bridge the gap between in vitro and in vivo work3. We developed a CRC spheroid that incorporates stromal cells, for assessment of the impact on cell viability and cell proliferation, as well as migration and invasive potential.

HCT116 and HT29 colorectal cancer cell lines were incorporated into collagen type I hydrogels and cultured alongside human MSCs (hMSCs) for 14 days to mimic the high stromal CMS4 CRC. Using Alamar blue and Cyquant, we have shown that cell proliferation and metabolic activity significantly increased from day 1-14. Using flow cytometry, we found that CRC cell lines composed the majority of proliferating cells in the gels. Calcein AM and Propidium Iodide stain were used to assess cell viability. Using ImageJ, we found that increasing the ratio of hMSCs in the culture led to a trend in increase in percentage viability of cells and a decrease in the percentage of dead cells. Introduction of an increased ratio of hMSCs also significantly increased cell outgrowth from spheroids, indicating increased invasive potential. These results indicate that the presence of hMSCs in CRC TME could play a role in viability of the CRC cells as well as assisting the metastatic ability of these cells. To further advance this model, we will incorporate these spheroids into a microfluidic device containing HUVECs as a vasculature-like component mimicking the increased angiogenesis in CMS4 CRC TME. Incorporating immune cells such as NK cells will further increase our understanding of cellular interactions taking place in the CRC TME. Having appropriate 3D models to assess immune and stromal impacts in CRC will increase knowledge of cellular interactions in the TME thus assisting in discovering novel target therapeutics.

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keywords: Colorectal cancer, 3D model, Tumour microenvironment

20941857249

ELUCIDATION OF COLLAGEN FIBRE STRUCTURE IN OSTEOGENESIS IMPERFECTA USING SECOND HARMONIC GENERATION IMAGING ON POLYCAPROLACTONE FIBRES

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Osteogenesis Imperfecta (OI) is an inherited connective disorder mostly caused by type I collagen (Col I) mutations resulting in low bone mass and increased fracture incidence [1]. Type I collagen is the structural protein that maintains the mechanical properties of bone. To date, analysis of collagen structure has been conducted using either patient bone biopsies or animal (mice) models.
This project aims to visualize an in vitro, human cell-based model of ECM deposition using electrospun Polycaprolactone (PCL) to understand how Col I mutations alter collagen structure in 3D. Second Harmonic Generation (SHG) has recently been used to visualize the extracellular matrix (ECM) of OI tissues [2]. PCL mats were fabricated by electrospinning into non-aligned and aligned fibers. Primary Human Dermal Fibroblasts (HDF) were obtained from PromoCell and OI HDF under informed consent from Sheffield's Children Hospital. Three different donors' cells denoted here 1, 2 and 3 were studied. All donors have Type I collagen mutation (COL1A1 gene). Donor 1 has type IV OI (qualitative defect), Donor 2 has type I OI (mild defect) and Donor 3 quantitative defect. Overall, in vitro collagen secretion by healthy and OI HDFs was determined by Sirius Red Assay. Cell-secreted collagen was structurally analysed using a Second Harmonic Generation (SHG) imaging on a laser scanning confocal microscope fitted with a Ti:sapphire laser. The results indicated that collagen secreted by healthy HDFs aligned in the direction of the electrospun PCL fibres [3]. Collagen secreted by OI fibroblasts cultured on aligned and nonaligned fibres also produced SHG signals but less strongly than healthy fibroblasts. The SHG signal produced by OI fibroblasts on aligned scaffolds are stronger and more elongated than nonaligned scaffolds and the collagen produced by OI HDF is less ordered. Sirius Red Assay supported the indication that OI HDF can secrete more collagen on aligned substrates.
Our results demonstrate that fibrous scaffolds can be used to create in vitro human cell based models in 3D. This will be a tool to better understand the mechanisms behind
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Acknowledgement: Tugba Cebe is funded by Republic of Turkey Ministry of National Education. Marie Skłodowska - Curie Actions: H2020 – MSCA – RISE - 2017 (777926). The project is funded by the European Union Nanosurf: R/153598

keywords: osteogenesis imperfecta, visualize the OI collagen, bio imaging, collagen orientation

83767248846

ENGINEERING A BIOMIMETIC THREE-DIMENSIONAL TUBULAR ORGAN-ON-CHIP USING SYNTHETIC BIOPOLYMERS TO MODEL PATHOPHYSIOLOGICAL CONDITIONS

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Introduction
Tubular tissues are ubiquitous in the body, from blood vessels and lymph ducts to bile and breast ducts. Evaluating drug efficacy for the treatment of pathophysiological conditions using in vitro models is crucial before initiating animal and clinical trials. A major problem with current in vitro models is the discrepancy between in vitro and in vivo results. This discrepancy exists mostly because the adopted in vitro models do not faithfully mimic the in vivo cellular and extracellular composition and dimensionality characteristics. Engineering a three-dimensional (3D) tubular model to mimic tubular tissues would be a significant step in reducing such discrepancies. We have manufactured a chip made of polymeric scaffolds to mimic the 3D architecture of tubular tissues. Currently, the model is being used to study breast and blood brain barrier tissues.

Methodology
Cell attachment: The chips were manufactured in the workshop and were covered with either collagen-I or poly-L-lysine. One milliliter of media with a cellular concentration of 300,000 cells per milliliter was placed on the chip. At different timepoints (0.5, 2 and 8 hours), the chips were washed with PBS and stained with DAPI/Calcein-AM to view and count the cells that attached.
Cell seeding: Cells were seeded in the channel at various densities (5, 10 and 20 million cells per milliliter) and monitored, with images taken on days 1, 4 and 7. On day 7, cells were stained with DAPI/Calcein-AM. Cell coverage of the channels was determined during the various days.
TEER measurements: Impedance spectroscopy was measured using a two-electrode setup with 2 Ag/AgCl electrodes. The impedance spectra were measured between 3 distinct points in the chip; the inlet, well, and the outlet.
Normal and cancerous breast cells co-culture: HMT-3522 S-1 and GFP labelled T-4 cells were co-cultured in the channel in a S-1:T-4 ratio of 10:1 to evaluate the interaction of these two cell lines. Cells were stained with DAPI and imaged after 7 days to monitor the growth of S-1 and T-4 cells.

Results
Cell attachment: Experiments showed that Collagen-I was a better attachment substrate than PLL.
Cell seeding: Seeding 20 million cells per milliliter proved most promising whereby cell coverage of the channel was adequate, and cells proliferated indicating metabolic activity. Lower seeding densities left vast areas of the channel lacking cellular coverage, even after 7 days in culture.
TEER measurements: TEER measurements showed that the impedance from either inlet/outlet to the center well were approximately half of the impedance from the inlet to the outlet of an empty chip. This measurement will allow us to investigate possible interferences of the channel structure on the TEER measurements to be carried out when complete cell coverage is achieved.
Normal and cancerous breast cells co-culture: HMT 3522 T-4 cells outgrew the S-1 cells significantly, with the vast majority of cells exhibiting GFP labelling.

Conclusion
The proposed tubular model shows promise to achieve biomimetic architecture of tubular tissues. This design would enable researchers to better evaluate the efficacy of drugs on tissues due to the improved biomimetic architecture.

keywords: Tubular architecture, LOC

41883634517

ENGINEERING BIOMIMETIC PATIENT DERIVED RENAL CELL CARCINOMA TUMOUROIDS

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Introduction:
Despite the significant advancements in the therapeutic management of renal cell carcinoma (RCC), there is still a pressing need for patient specific platforms that can predict personalised treatment response. Tumouroids are tissue-engineered 3D in vitro models that mimic the physiological tumour microenvironment, can reproduce cell-cell and cell-matrix interactions and incorporate patient derived tumour material.
Our aim was to manufacture compartmentalised biomimetic renal cancer tumouroids using collagen type I, that incorporate primary cells isolated from RCC surgical specimens in the inner compartment, surrounded by a stromal compartment that recapitulates the extracellular matrix (ECM) density, composition and cell populations of the in situ tumour microenvironment. We hypothesized that tumouroids will recapitulate the original tissue in terms of phenotype, genotype and response to treatment.

Methods:
RCC samples were collected post-surgery (n=16), different tissue disaggregation methods were used and the isolated cells were grown in 2D and tumouroids. Tumouroids were manufactured either as simple (one compartment, populated with primary RCC cells) or complex (two compartments: a simple tumouroid as the inner compartment surrounded by a stroma compartment populated with fibroblasts and endothelial cells). RAFT absorbers were used to remove excess liquid to increase the matrix density reproducibly, to near human cancer ranges. Immunofluorescence and confocal microscopy were used to determine whether the patient derived tumouroids retain the expression of RCC markers and whole exome sequencing (WES) was performed to investigate resemblance to the parental tumour. Cell proliferation was compared among different culture conditions by measuring ATP production. Tumouroids were also challenged with a clinically used tyrosine kinase inhibitor, pazopanib.

Results:
We showed that the patient derived RCC tumouroids, made by cells isolated via different methods, retain the expression of renal cancer associated gene markers, including mutant VHL and characteristic protein markers, including cytokeratins CK8/18 and alpha smooth muscle actin (αSMA). Non linear increase in proliferation was observed in the tumouroids which may be indicative of 3D organization, differentiation and other cellular functions. Tumouroids' response to pazopanib ranged from none to strong (40%). Additionally, when treatment response was compared among tumouroids of different levels of complexity, tumouroids that incorporated a stromal compartment responded more than simpler tumouroid models (n=3).

Conclusion:
Our results indicate that RCC cells maintain their phenotype and genotype when grown as tumouroids. Future work will compare the response from tumouroids to the responses from both xenografts and the actual patients to determine the suitability of tumouroids as

personalised cancer treatment platforms.

keywords: 3D in vitro models, renal cell carcinoma, tumouroid, biomimetic, microenvironment

20941859087

GLIOMA-ON-A-CHIP PLATFORM

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Introduction: Glioma is known as one of the most malignant brain tumors originated from glial stem cells or progenitor cells [1]. Current therapies to cure glioma cases still seem insufficient [2] due to glioma's invasiveness and heterogeneous tumor microenvironment [3]. To investigate glioma behavior thoroughly and to develop personalized therapy for tumors is challenging, and various 3D microfluidic platforms were proposed to study glioma tumors [3]. Recently, natural or synthetic polymers have been used for creating the 3D environment for cells. Here, we proposed a microfluidic platform that mimics the glioblastoma tumor microenvironment. We integrated the gelatin-derived hydrogel and microfluidic chips to obtain a more realistic glioma tumor microenvironment.

Methodology: Novel multiplexed microfluidic chips were designed and fabricated via uv&soft lithography. The photomasks were specifically designed and produced to create desired hydrogel geometry within the chips. Prepolymer solution was prepared by adding 5% methacrylated gelatin (GelMA) and 1% Irgacure 2959. Hydrogel contained photoinitator was mixed with U373 glioblastoma cells, and they were transferred into the microfluidic chips by syringe pump. Encapsulation of the glioblastoma cells (U373 human glioblastoma) within the GelMA hydrogel was performed by photocrosslinking under the 365 nm of UV light, and specially designed photomasks was put on the chip during this process. Cell viability, after encapsulation, were analyzed by cell toxicity LIVE/DEAD assay.

Results: Our studies demonstrated that GelMA hydrogel in the microfluidic chip reservoirs demonstrate sufficient structural integrity under the shear stress due to perfusion. High cell viability of encapsulated U373 glioblastoma cells within the GelMA in the chip was observed after several days.

Conclusion: Current therapies for glioma have challenges due to glioma's invasive character. A depth understanding of its molecular mechanisms is essential to develop personalized treatment. Mimicking tumor microenvironment via microfluidic chips has high potential to create a more realistic in vitro glioma tumor environment, leading to study cell migration, invasion, angiogenesis, and patient-specific treatment [3]. Moreover, in vivo animal studies would be eventually replaced with microfluidic platforms, and these also platforms allow investigating the drug combinations. In this study, we developed microfluidic platforms that offer a high chance of examining glioma tumors and investigating personalized therapies.

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Gelatin methacryloyl (GelMA) was purchased from Zetamatrix Biotechnology.

keywords: microfluidic chips, biomaterials, GelMA, glioma, 3D cell culture

62825424647

IN VITRO 3D OSTEOSARCOMA MODELS TO IMPROVE THERAPY OUTCOMES TOWARDS CANCER STEM CELLS NICHE

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Introduction. Worldwide cancer remains the second-most common cause of death; among bone cancers, Osteosarcoma (OS) is the most common type diagnosed especially in children and young adults1. The lack of specificity for the Cancer Stem Cells (CSCs) subpopulation has been recently identified as the main limitation in conventional therapies2. Moreover, the traditional two-dimensional (2D) in vitro models, employed in the drug testing as well as in biological studies, are affected by a poor in vitro-in vivo translation ability3. This work provides "tumour engineering" osteosarcoma models as new tools to address the disease and improve therapy outcomes.

Methodology. Two different hydroxyapatite-based scaffolds4,5 were used to recapitulate in vivo bone extracellular matrix (ECM); a ceramic scaffold from a direct foaming process and a hybrid biomineralized scaffold were used.
MG63 and SAOS-2 osteosarcoma cell lines were used as parental cells and spheroids. As well-established CSC-enrichment model, the sphere-forming culture6 was used to obtain spheroids under serum free and ultralow-attachment conditions.
The 2D in vitro preliminary evaluation of spheroids and parental cells to confirm CSCs enrichment was performed by gene expression of stemness markers. The phenotype of spheroids under 3D scaffold-based models were investigated by histological, fluorescent and electron microscopy analysis compared to those with parental cells. Stemness (OCT-4, NANOG and SOX-2) and CSC niche-related genes (NOTCH-1, HIF-1α and IL-6) and proteins expression was analysed on spheroids under 3D scaffold-based conditions compared to 2D ones.
Actually, the variability of tumoral properties in serial spheroids passaging7,8 is still being investigated; spheroids serial generations of osteosarcoma cells have been characterized by proliferation, sphere-forming efficiency, migration and invasion ability, and gene and protein expression profile under 2D conditions.

Results. The enrichment of a cell population with stemness properties was confirmed by 2D analysis of gene expression. The morphological evaluation of in vitro 3D scaffold-based models highlights the maintenance of spheroidal phenotype by spheroids. The gene and protein expression confirmed the presence of stemness and CSC-niche markers by 3D scaffold-based spheroids compared to 2D conditions, underling the importance of three-dimensional

microenvironments for in vitro studies.
The variability of serial passaging spheroids of osteosarcoma cells was partly reported, giving a starting point to select the right spheroids generation to furtherly improve our tumour models. Moreover, tumour matrix complexity, co-cultures and specific secretome will be included as part of tumour microenvironment.

Conclusions. These 3D in vitro tumour models could improve the predictivity of preclinical studies and enhance the clinical translation, with the ultimate goal to be applied in personalized medicine.

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keywords: Tumour Engineering, osteosarcoma, in vitro 3D tissue modeling, scaffolds, spheroids

73296315055

LABEL-FREE FLUORESCENCE LIFETIME IMAGING MICROSCOPY AND RAMAN MICROSCOPY FOR IN SITU DRUG EFFICACY TESTING ON PATIENT-DERIVED BLADDER CANCER ORGANOIDS.

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Three-dimensional organoid culture offers great promise for precision medicine in cancer. In this study, we describe label-free imaging procedures including Fluorescence Lifetime Imaging Microscopy (FLIM) and Raman microscopy allowing in situ cellular analysis and metabolic monitoring of drug treatment efficacy. Primary tumor as well as urine specimens were utilized to assemble a biobank of bladder cancer organoids. Organoids, recapitulating histopathological and molecular diversity were cultured in Matrigel and treated for several days with various concentrations of pharmaceutical agents relevant for treatment of bladder cancer (i.e. cisplatin, venetoclax). The effects of different drugs on cell metabolism were assessed by the local fluorophore environment of NADH and FAD determined by multiexponential fitting of lifetime decays. Direct cellular response upon drug treatment was monitored by Raman microspectroscopy. Via multivariate data analysis, Raman spectra of treated and untreated bladder cancer organoids were compared to reveal the effects of drugs on subcellular structures such as nuclei and mitochondria based on shifts and intensity changes of specific molecular vibrations. Drug-specific modes of action were reflected and discriminated in the metabolic profiles and molecular composition of the tumor models. Together, FLIM and Raman imaging are technologies allowing non-invasive and molecular-sensitive monitoring of tumor-drug interactions, providing the potential to determine and optimize patient-specific treatment efficacy.

keywords: Cancer organoids, Raman spectroscopy, Fluorescence lifetime imaging microscopy, Drug screening

94238169517

MATRIX REMODELING DURING PANCREATIC CANCER CELL ORGANIZATION

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Introduction: Pancreatic cancer has the lowest survival rate among the common cancers, mainly due to its symptom-free progression and the advanced stage of the disease at the time of diagnosis. The response to current treatment strategies is low, mainly due to the extensive desmoplasia and fibrotic extracellular matrix (ECM), which acts as a barrier against efficient drug delivery.
One distinctive feature of the pancreatic tumor microenvironment (TME) is the extensive remodeling and stiffening of the matrix, mainly due to the excessive production of ECM proteins [1]. 3-dimensional (3D) in vitro platforms proved to be valuable tools in studying the dynamics of TME and its remodeling during disease progression. However, current 3D in vitro models of pancreatic cancer usually overlook the dynamics of matrix remodeling during the autonomous cell organization [2]. This study aimed to evaluate the dynamics of matrix remodeling during the growth and autonomous organization of pancreatic cancer cells in a protease-sensitive and degradable hydrogel matrix based on starPEG-Heparin with defined biochemical and physical properties [3].

Methodology: The starPEG-Heparin hydrogels were designed to provide adequate initial stiffness according to the published datasets from patient-derived tissues [2]. The incorporation of protease-sensitive peptides provided the dynamic degradability of hydrogels throughout the 3D culture. RGD-peptide sequences were incorporated into the hydrogel matrix, enabling integrin-mediated cellular adhesion. Oscillatory shear measurements and atomic force microscopy (AFM) were used to evaluate the mechanical properties of the hydrogels. Pancreatic cancer cells, together with stromal cells including cancer-associated fibroblasts (CAFs) and immune cells, were grown encapsulated into hydrogels and cultured for 14 days. The dynamics of stiffness remodeling in these multicellular cultures were evaluated by AFM and compared to mono-cultures containing only pancreatic cancer cells.

Results: The initial stiffness of the hydrogel matrices was set to be in a range with the median value of ~15 kPa, by changing the degree of crosslinking. AFM data showed that while hydrogels with mono- and multicellular-cultured cell populations have comparable stiffnesses at the beginning of the culture period, both cell populations contributed to a progressive stiffness reduction after 14 days of culture. Cells organized themselves autonomously within the hydrogels by forming spheroids, followed by the degradation of the hydrogel matrix. However, the size and the number of spheroids in mono-cultures were significantly less than in multicellular cultures. Compared to the initial matrix, the extent of remodeling was significantly less in mono-cultures. Notably, the stiffness of the matrix distant from spheroids in multicellular cultures was significantly lower after 14 days of culture compared to the beginning of the culture period. In contrast, the stiffness in the vicinity of spheroids was considerably larger, suggesting the production and accumulation of newly synthesized ECM.

Conclusions: The spontaneous organization of cancer cells in hydrogels to form cellular aggregates is observed in many in vitro 3D cancer models. Our results suggest that remodeling degradable starPEG-Heparin hydrogels by pancreatic cancer cells is a heterogeneous process. In the presence of stromal cell populations, the intracellular interactions could produce newly formed ECM, profiling the stiffness of cancer tissue.

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keywords: pancreatic cancer, starPEG-heparin hydrogels, matrix remodeling, tumor microenvironment

52354542129

MICROPARTICLE BASED MICROGEL FOR THE STUDY OF THE TUMORAL MICROENVIRONMENT (TME).

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Background: Tissue engineering methodologies have been used to generate in vitro models that emulate the characteristics of different types of cancer, including lung cancer. Lung cancer stands out for its great capacity for dissemination and mortality, based in part on the great capacity of cancer cells to develop drug resistance and in part on the complexity of tumor microenvironment (TME). TME includes fibroblasts, cancer-associated fibroblasts (CAFs), endothelial cells, and the extracellular matrix components. The interactions between they determine the characteristic behavior of lung cancer. We propose to generate a three-dimensional model for the in vitro study that allows us to better understand the interaction between CAFs, non-tumor fibroblasts and cancer cells. This system will allow not only to identify new possible pharmacological targets, but also to increase our knowledge regarding to the acquisition of drug resistance or the metastasis process.

Methods: Alginate microspheres with a diameter around 250 μm were produced by microfluidics. The surface was coated with two bilayers of gelatin/alginate polyelectrolyte multilayer using a layer-by-layer, LbL, procedure. A covalent cross-linking of the coating was performed with glutaraldehyde followed by a treatment with glycine to inactivate remnant unreacted aldehyde groups. CAFs and non-tumor fibroblasts were isolated from 4 human lung cancer tumors. Cells were characterized by flow cytometry according to CD29, CD31, CD44, CD45, CD73, CD105, CD146, and STRO-1 expression. The cells were cultured with microparticle in DMEM cultured medium supplemented with 10% fetal bovine serum, penicillin, streptomycin and fungizone for 72 hours under orbital shaking. Agarose-coated cell culture wells were used to prevent cell adhesion to the plastic of the culture plates. Cell adhesion and morphological characteristics of CAFs and non-tumor fibroblasts were studied by fluorescence staining of F-acting using phalloidin-rhodamine. Vimentin, FSP1, type I collagen, and N-cadherin expression were analyzed by immunofluorescence and real time RT-PCR.

Results: The isolated cells were positive for CD29, CD44, CD73, CD105 and CD146, and negative for CD31, CD45 and STRO-1. Significative differences in the expression of CD45 (18.45 ± 2.14 positive CAFs compared to 8.27 ± 1.15 positive non-tumoral fibroblasts) were found. After 72 hours of culture, the cells adhered to the microparticles. These cells showed a stellate morphology, with abundant actin stress fibers. Vimentin and FSP1 expression was significantly higher compared to that of cells cultured in the absence of microparticles. Likewise, a marked expression of type I collagen by the cells cultured on the microspheres was detected. No significant differences were observed between CAFs and non-tumor fibroblasts in relation to the proteins studied.

Conclusion: The manufactured microparticles represent a suitable support for the culture of stromal cells isolated from lung cancer tumor tissue. The cells that adhered to the microparticles

acquired a more marked secretory mesenchymal phenotype compared to that cells cultured in conventional 2D systems. This system will allow the generation of co-culture systems of CAFs and non-tumor fibroblasts in different ratios in a controlled manner, being a suitable in vitro system for the study of the effects of different drugs on stromal cells of TME.

keywords: lung cancer, microgel, organoids, tumor extracellular matrix, tumor microenvironment

52354541048

MODELLING IDIOPATHIC LUNG FIBROSIS IN VITRO: A NOVEL STRATEGY FOR THE ASSESSMENT OF ANTI-FIBROTIC PHARMACEUTICALS

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Idiopathic pulmonary fibrosis (IPF) is a degenerative and fatal disease, characterised by scarring of lung parenchyma and loss of respiratory function. Globally, this disease affects approximately three million people, and carries a typical life expectancy of 3-4 years at the point of diagnosis [1]. IPF is now considered to arise as a result of chronic damage to the alveolar epithelium, followed by pathological activation of underlying fibroblasts, excessive ECM deposition and remodelling, and the gradual stiffening of lung tissue [2]. The aetiology of IPF remains largely unknown, although several risk factors have been identified. Emerging data from the ongoing COVID-19 pandemic has highlighted a potential link between coronavirus infection and pulmonary fibrosis. Acute respiratory distress syndrome (ARDS), a form of respiratory failure, is often followed by pulmonary fibrosis. Early studies estimate 40% of patients presenting with COVID-19 pneumonia develop ARDS [3]. These patients are therefore at risk of developing pulmonary fibrosis as a long-term consequence of infection. Heavy reliance on in vivo animal models of IPF has impeded the discovery of effective therapeutic compounds, due to significant differences in animal and human pathophysiology. In human IPF patients, fibrosis is a chronic degenerative disease that occurs over a long period of time in response to a prolonged series of low-grade injuries. Most animal models however, represent an accelerated acute fibrotic response, linked to inflammation and in response to a single high-grade stimulus [2,4]. Such models therefore have limited pre-clinical success for screening therapeutic compounds. There remains a need for more representative, humanised models of IPF.

We have developed a novel in vitro construct that can incorporate both epithelial and interstitial fibroblast compartments to better recapitulate aspects of the alveolar epithelial tissue. These constructs utilise a porous, polystyrene scaffold membrane to support the growth of healthy and IPF-derived pulmonary fibroblasts, and thereby enable endogenous ECM deposition. Such models can be used for the assessment of anti-fibrotic compounds and their ability to modify ECM with the stomal compartment. Alveolar epithelial cells can also be seeded onto the underlying fibroblast compartment to form a more complex, full thickness model of the alveolar epithelium.

Quantification of endogenous collagen and fibronectin proteins within bioengineered constructs have shown an innate characteristic of IPF-derived fibroblasts to secrete and deposit greater concentrations of ECM fibres, relative to healthy lung fibroblasts. Incorporation of an overlying epithelial compartment has enabled the development of a more complex and more physiologically representative in vitro model of IPF. Such models can be treated with appropriate stimuli to mimic low grade chronic injuries to the alveolar epithelium which subsequently activate the underlying fibroblast compartment and further induces fibrosis.

These results highlight the potential for this novel bioengineered construct to be used for the assessment and effectiveness of anti-fibrotic compounds using in vitro assay analysis.

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keywords: IPF, Respiratory, Fibrosis, ECM

31412780379

MODELLING INFLAMMATORY BOWEL DISEASE IN VITRO FOR THE DEVELOPMENT OF PHARMACEUTICALS

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Ulcerative colitis (UC) and Crohn's disease (CD), collectively known as inflammatory bowel diseases (IBD), are chronic inflammatory disorders of the gastrointestinal tract. IBD is an idiopathic disease characterised by a dysregulated immune response that results in mass-immune cell infiltration and subsequent tissue damage. The mechanisms underpinning the complex pathogenesis of IBD requires further elucidation.

Current therapeutic strategies in IBD primarily target activated leukocytes in order to dampen the immune response, neglecting other critical mediators of inflammation, such as fibroblasts. There now exists irrefutable evidence that fibroblasts are not just bystanders in mucosal inflammation but are pivotal participants in orchestrating the immune response in IBD.1 Although more recent therapies such as biologic agents have shown more success in treating IBD than previous immunosuppressive drugs, 40% of patients elicit no response. Moreover, approximately 90% of clinical trials for investigational new drugs (IND) fail, mainly attributable to the fact that most IND research is carried out in animal models, ex vivo-tissue and oversimplistic in vitro culture methods.2 The scarcity, disease-severity and short-lived nature of IBD tissue ex vivo, low similarity between animal and human tissue, and absence of physiologically relevant in vitro models impedes research progression and subsequent drug development.3,4

We have bioengineered highly reproducible, in vitro models that recapitulate key aspects of the inflamed IBD intestinal mucosa. These constructs utilise a porous polystyrene scaffold membrane to construct lamina-propria-like compartments with an overlying epithelium. Subepithelial fibroblasts within the lamina propria secrete endogenous extracellular matrix (ECM), alongside a co-cultured immune component that simulates the mass immune-cell infiltration observed in IBD. An inflammatory phenotype is induced through addition of appropriate inflammatory stimuli. Inflammatory parameters characteristic of IBD are observed, including endogenous inflammatory cytokine and chemokine secretion, barrier impairment, and ECM remodelling. This in vitro system allows for investigation into complex interplay between epithelial, mesenchymal and immune cells and the resultant effects on barrier function, thus simulating key aspects of IBD.

Through treatment with relevant and well-characterised therapeutic compounds, we further demonstrate the functional potential of the mucosal constructs in obtaining drug efficacy data. Using both prophylactic and therapeutic approaches, we are able to generate datasets that would otherwise be impossible to obtain using ex vivo IBD tissue due to the short-lived nature of ex-vivo assays. This subsequently allows for dissection of the anti-inflammatory effects of the therapeutic compounds within models by analysing changes in inflammatory parameters.

Taken together, our results demonstrate the potential for this novel, bioengineered system to be used for pre-clinical pharmaceutical compound testing in a controlled, reproducible and physiologically relevant environment.

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keywords: Intestine, IBD, Inflammation, Pharmaceuticals

83767243897

NOVEL MECHANICALLY TUNEABLE THREE-DIMENSIONAL IN VITRO MODELS FOR PROSTATE CANCER PROGRESSION AND ADAPTATION UNDER DIFFERENT STIFFNESS

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Prostate cancer is the most life-threatening cancer for men with high incident and mortality rates, accounting for 1.4 million cases worldwide in 2020 with around 52,000 new cases in the UK every year. The progression and malignancy of prostate cancer have been linked with enhanced fatty acid utilisation that may be driven by the need of the cancer cells for energy to support growth and metastasis. The development of new early-detection biomarkers and therapeutics for prostate cancer has been hindered by the lack of preclinical systems able to model the human tumour microenvironment. Growing cells in traditional two-dimensional (2D) cultures, cannot recapitulate essential tumour characteristics such as cell-cell and cell-matrix interactions that are observed in tissues' three-dimensional (3D) organization. Additionally, 2D cultures cannot model the stiffness of prostate tissue, a property linked to disease development. To overcome these challenges 3D culture models have been developed with a view to enhance the complexity of in vitro models and provide a more realistic cell culture system.
In this project, we have used alginate-based hydrogels and formulated them to match the physical and chemical properties of the prostate tumour microenvironment, in particular the stiffness and extracellular matrix (ECM) composition. To develop this model, we used three prostate cell lines: the prostate epithelial cells PNT-2, the bone-metastatic prostate cancer cells PC-3, and the lymph node-metastatic prostate cancer cells LNCaP. Cells were encapsulated in alginate-gelatin-laminin hydrogel beads, formulated to have different physical properties. Soft hydrogels containing alginate (0.25% w/v), gelatin (3% w/v), and laminin (10 μg/mL) were designed to mimic physical properties of normal prostate microenvironment, whereas stiff hydrogels containing alginate (0.75% w/v), gelatin (3% w/v), and laminin (10 μg/mL) mimicked physical properties of prostate tumour microenvironment. The stiffness of the hydrogels was tested at different crosslinking density and set to 1.4 kPa for soft hydrogels and 6.9 kPa for the stiff ones. Prostate cells were encapsulated into hydrogel beads at an initial density of 1x106 cells/mL; proliferation and metabolic activity were measured up to 7 days. Whilst the metabolic activity of the prostate cancer PC-3 and LNCaP cells was reduced when encapsulated in stiff hydrogels, it was found to continuously increase in soft hydrogels. Conversely, the prostate epithelial PNT-2 cells did not show any change in their metabolic activity as function of hydrogel stiffness.
Our results show that prostate cancer cells behave differently in soft and stiff 3D microenvironments and this could be indicative of cellular adaptation. Detailed characterisation of relevant cellular biomarkers is needed to further understand the cells' response to the altered microenvironment. The results of this study would provide novel insight into the prostate cancer cell adaptation to normal and tumour microenvironments. Further development and validation of this 3D in vitro model would help to understand the biochemical changes that occur during prostate cancer progression and could support the discovery of novel biomarkers and therapeutics.

keywords: Prostate cancer, 3D culture, in vitro model, lipid metabolism

31412706027

THE EFFECT OF MACROMOLECULAR CROWDERS ON DEPOSITION OF EXTRACELLULAR MATRIX IN ASTROCYTES

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Introduction: Spinal cord injury (SCI) is a condition that has caused disability worldwide. However, progress in the development of effective therapies to treat SCI-associated neuropathologies has been slow so far. In particular, a few days after injury, a scar made of two components, an outer glial scar and an inner fibrotic scar composed of extracellular matrix (ECM) proteins, is formed and some inhibitory ECM molecules become upregulated. Changes in the composition and structure of the ECM primarily contribute to regeneration failure in the injured spinal cord. Hence, developing model systems that enable cells to produce ECM within in vitro assays is a critical need to test therapeutic strategies for targeting inhibitory molecules. One proposed strategy is to expose cells to an environment crowded with adequate macromolecules to mimic the physiological cellular milieu. In this study, astrocytes were cultured in the presence of macromolecular crowders (MMC) which can dramatically improve the deposition of a variety of ECM molecules. The ECM deposition can be modulated by the biophysical properties of the crowder. Herein, we assessed the influence of different concentrations of MMCs, dextran sulphate (DxS), and carrageenan (Ca), on Neu7 astrocyte cell morphology, metabolic activity, viability, and collagen type I (Col I) deposition. Furthermore, the effect of optimized MMCs concentration on the expression of different ECM proteins of the glial and fibrotic scar was investigated.

Methodology: Cells were cultured in DMEM-low glucose supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin. The cells were seeded at a density of 25,000 cells/cm2 in the conventional medium. After 24 hours, the medium was replaced with the same conventional medium (control group) and medium with 100 mM ascorbic acid and different concentrations of DxS and Ca. Cells were grown for 5 days in each condition. Phase-contrast images were captured using an inverted microscope at different time points (3 and 5 days) to evaluate the influence of DxS on cell morphology. In addition, the alamarBlueTM assay was used to assess the effect of DxS on cell metabolic activity. On day 5, the viability of cells was evaluated by Live/Dead staining and then analyzed by fluorescence microscopy. Immunocytochemistry (ICC) was performed to assess deposition of Col I and other proteins of the glial and fibrotic scar, such as glial fibrillary acidic protein (GFAP), chondroitin sulphate proteoglycan proteins (NG2 and CS56), fibronectin, and collagen IV.
Result and Conclusion: Phase contrast images revealed that MMCs did not have any impact on cell morphology, and the cells maintained their spindle-shaped morphology at all time points. Additionally, the metabolic activity and cell viability of cells cultured in medium with MMCs were identical to those cultured in conventional medium. ICC analysis demonstrated that at 100 mg/ml MMCs, the Col I deposition significantly increased compared to no MMCs. Ca and DxS enhanced the accumulation of cell-secreted molecules which formed a secreted matrix. This novel culture system opens up a new avenue to the screening of therapeutic compounds to

modulate the environment of the glial and fibrotic scar in spinal cord repair.

keywords: Spinal cord injury, glial/fibrotic scar, Extracellular matrix, macromolecular crowders

73296338946

THREE-DIMENSIONAL CELL CULTURE SYSTEM AS AN IN VITRO PLATFORM FOR LUNG CANCER MODELING

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Introduction
Non-small cell lung cancer (NSCLC) is a widespread tumor classified in heterogeneous subtypes according to the cell of origin and molecular features (1). Disease progression is linked to complex microenvironment, which includes several cell types, matrix components (2) and pervasive angiogenesis (3). Traditional 2D culture fails at resuming the complexity of in-vivo model, whereas 3D environment (e.g. scaffolds) sustains cell culture with stimuli alike the physiological ones (4). The aim of the work is the development of an in-vitro device able to mimic the NSCLC niche and using it as more reliable platform for molecular studies and drug screening.

Methodology
Methacrylate gelatin (GelMA) (5), is used as hydrogel for the establishment of long term cell culture. To mimic NSCLC features, two adenocarcinoma cell lines, A549 and H1299, and normal lung fibroblast MRC-5 are selected. Cells at the density of 1,5x106 cells/ml are dispersed into the formulation and photopolymerized at l=405 nm. Then, biocompatibility of different GelMA hydrogel, are analyzed by MTT assay, calcein AM/PI staining and cytofluorimetric analysis.

Results
Three different GelMA formulations (medium, high and very high), were obtained by modulating its degree of substitution. Methacrylation level and GelMA percentage determine pore size and matrix stiffness, which, in turns, influences cell behavior (6). Consequently, several combinations were tested to identify the preferable culture conditions. Since the aim of the work is the development of scaffolds which resemble tumor microenvironment features, A549 and H1299 cells were grown as mono-culture or co-culture with normal fibroblasts (MRC-5) to determine the importance of the cross-talk between these two cell types. Particularly, two ratios tumor : fibroblast were chosen: 1:1 and 1:2. Then, MTT analysis are performed after 1 – 6 - 14 days of culture. For A549 model, high 10% and very high 10% result the better matrices whereas, for H1299, the percentage must be increased at 12,5%. CalceinAM/PI staining revealed good cell viability at 6 - 14 days, confirming the absence of matrix toxicity. Moreover, the long term ratio of the two cell types was verified by cytofluorimetric analysis.

Conclusions
Our results support the feasibility of using GelMA as matrix for 3D long term cell culture. Further analysis will be done to dissect the crosstalk that interplays between the two cell types and the matrix, which will be compared with the one of in-vivo model. Moreover, since GelMA is a promising ink for 3D-bioprinting, the system will be implemented by the introduction of a 3D printed-microfluidic, which will mimic the physiological vasculature.

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keywords: Lung cancer, Extracellular matrix, Bioprinting

83767214319

TOWARDS A NEW THERAPEUTIC MODALITY FOR BLADDER CANCER: VECTORISED PHOTODYNAMIC THERAPY VALIDATED IN TUMOR MODELS OF INCREASING COMPLEXITY

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Introduction. Photodynamic therapy (PDT), an anti-cancer therapeutic approach based on the selective activation by light of photosensitive molecules called “photosensitizers” 1, is promising in the context of bladder cancer treatment. This is due to the accessibility of the tumors in the bladder cavity for both instillation of photosensitizer and light irradiation 2. A main drawback of photosensitizers is their low aqueous solubility that results in their aggregation and which lower the PDT efficacy. In order to improve their delivery and therapeutic efficacy, we propose to encapsulate photosensitizers in nanocarriers based on self-assembled polymer micelles 3. We aimed at understanding and quantifying how photosensitizers, whether or not vectorised in polymeric nano-objects, behave at bladder tissue scale in the perspective of proposing a safe, efficient and easy to handle treatment against human bladder cancer.

Methodology. In the context of this work, pheophorbide a was used as model photosensitizer 4. It was encapsulated within self-assembled polymer micelles based on poly(ethylene oxide)-block-poly (ε-caprolactone) PEO-PCL, an amphiphilic block copolymer 5. Efficacy of the PDT was tested on a panel of bladder tumor cell lines of different degree of aggressiveness, namely T24, SW780, SW1710 and MgHu3. Moreover, the treatment was performed in tumor models of increasing complexity: 2D monolayer, 3D tumor spheroids and human cancerous bladder substitutes produced by tissue engineering using the self-assembly approach 6.

Results. The encapsulation of pheophorbide a in PEO-PCL micelles resulted in an improvement of PDT efficiency in both 2D monolayer and 3D tumor spheroids, yielding a 10-fold improvement of the therapeutic index for the same pheophorbide concentration. Two-photon microscopy observations revealed increased tissue penetration of the pheophorbide within the tumor spheroids when it was encapsulated within micelles compared to its free form. Experiments in human 3D bladder cancer substitutes are still ongoing and will help to better describe tumor cell response to PDT within a complex microenvironment.

Conclusion. Vectorization of hydrophobic photosensitizers undoubtedly increases its diffusion capacity within tumor tissue, ensuring a better therapeutic efficacy when stimulated with light during photodynamic therapy.

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keywords: Photodynamic therapy (PDT), bladder cancer, drug delivery, polymer micelles

20941823044

TYPE VII COLLAGEN EVALUATION IN RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA HUMAN SKIN EQUIVALENTS FOLLOWING NON-VIRAL VECTOR GENE THERAPY

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Introduction
Currently, human skin equivalents (HSEs), used as 3D in vitro models, are a particularly attractive intermediate step between conventional cultures and in vivo experimentation. Our group is focused on the development of non-viral gene therapies, based on hyperbranched poly (β-amino esters) (HPAEs), for Recessive Dystrophic Epidermolysis Bullosa (RDEB). Traditionally, RDEB-HSEs have been used to evaluate ex vivo therapies by confirming that edited keratinocytes and fibroblasts are capable of forming a healthy skin with collagen VII (C7) restoration in the basement membrane zone (BMZ) and lack of blister formation. In this work, we have studied the use of RDEB-HSEs as a method to evaluate topical gene therapies efficacy for potential in vivo RDEB treatments. Transfected HSEs at different time points of their development with different treatments and topical formulations have shown recombinant C7 expression in the BMZ directly related with skin penetration and indirectly with stratum corneum development.

Methodology
Nanoparticles, called polyplexes, where formed by combination of HPAEswith a full COL7A1 cDNA as described in Wang et al., 2020 (1). Efficacy of polyplexes was tested in conventional cultures of RDEB keratinocyte (RDEBK) and after 48 hours C7 was evaluated by Western-blotting and immunofluorescence. RDEB-HSEs were performed based on the ones described in Gache et al., 2004 and LLames et al. 2004, by increasing fibrinogen and aprotinin by 25%, doubling the ascorbic acid concentration and adding extra medium volume in the well when seeding the keratinocytes to avoid medium diffusion in the transwell (1,2). Test treatments applied topically after 4, 8, 12 and 16 days of maturation. Polyplexes were prepared in two buffer systems (H2O or NaOAc) and applied fresh or after lyophilization with jojoba oil. 48 hours post-treatment, HSEs were embedded in O.C.T. medium and snap frozen in liquid nitrogen, 7 µm sections were stained with anti-C7 antibody hLH 7:2pAb and mounted with mounting media with DAPI for immunofluorescence analysis (3). Microphotographs were taken on an Olympus CKX41 inverted microscope using a monochromatic camera (Q-Imaging) and CellSens imaging software (Olympus). Image post processing and analysis were performed by using ImageJ (NIH) open-source software.

Results
Transfection in conventional in vitro culture of RDEBK induced 10-fold higher C7 formation compared with normal human keratinocytes. The generated HSEs showed a good microscopical structure, however, no significant differences were noted when using increased concentrations of fibrinogen and aprotinin compared to standard ones. Transfection was not successful at 16 days for any of the test treatments, most likely due to impaired cellular uptake of the treatment

which is in contrast to in vitro transfection of keratinocytes at 48h after seeding, proving to be highly effective in producing C7. Decreased transfection could be a direct cause of keratinocyte terminal differentiation and stratum corneum formation, a well-known barrier for external agents despite our created defect.

Conclusion(s)
RDEB-HSEs are a very good method to validate formation of restored skin when pre-treating the cells, however multiple challenges need to be overcome to use the matured skin equivalents as validated method to evaluate efficiency of topical gene therapies.

keywords: COL7A1, RDEB Human Skin Equivalents, Gene Therapy, Hyper-Branched Poly (β-Amino Ester)

PS02
3D in vitro tissue-engineered cancer/disease models – Session II

31412744877

CHARACTERISATION OF COLLAGEN/CHONDROITIN SULFATE AND COLLAGEN/HYALURONIC ACID SCAFFOLDS TO MODEL THE PROSTATE CANCER MICROENVIRONMENT.

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Characterisation of Collagen/Chondroitin Sulfate and Collagen/Hyaluronic Acid Scaffolds to Model the Prostate Cancer Microenvironment.

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Prostate cancer (PC) is one of the leading causes of cancer-related death in men worldwide.
In the early stages of advanced PC, cancer cells undergo epithelial-to-mesenchymal transition (EMT), a process whereby epithelial cells lose their cell-cell adhesion, gain migratory and invasive characteristics, and travel through the blood vessels to invade new tissue, leading to metastasis. Approximately 90.1% of those who die with PC have bone metastases. Studies suggest that combining Docetaxel with hormone therapy helps in reducing PC progression and spread but ultimately only prolongs the length of survival. As a result, there is a major unmet need to develop alternative treatments. The extracellular matrix (ECM) of prostate tissue contains various constituents, including chondroitin sulfate (CS) and hyaluronic acid (HyA), which are significantly increased in metastatic PC and may be of interest for further investigation. This project aims to develop three-dimensional (3D) porous collagen/CS (Col/CS) and collagen/HyA(Col/HyA) scaffolds suitable for studying in vitro PC EMT and as a platform to test and develop treatments for PC.
Three Col/CS scaffold compositions containing 0.5 weight/volume (w/v) collagen and 0.05, 0.55, and 1.05 w/v CS were prepared. Additionally, two Col/HyA scaffold compositions containing 0.5 w/v collagen and 0.05 and 1.05 w/v HyA using freeze-dryer technique. These scaffolds were subjected to rigorous scaffold characterisation, including mechanical testing, SEM, pore size analysis, and porosity measurements. To determine the impact of CS and HyA on PC EMT processes and metastasis growth, osteolytic PC-3 and osteoplastic LNCaP PC cells were cultured on all scaffolds for biochemical evaluations, such as metabolic activity, DNA quantitation, and Live/Dead cell viability assays. Additionally, EMT cytokines expression was examined on the scaffolds using Cytokine Array kit to determine the impact of increasing CS and HyA concentrations on EMT cytokines expression.
Overall, the highly porous scaffolds were successfully developed. The stiffness significantly increased after EDAC cross-linking for all Col/CS, and Col/HyA low but all scaffold compositions were less stiff than PC tumours. All the scaffolds swelled at a high rate and remained stable and free of deformity. All scaffolds supported PC-3 and LNCaP cell growth and proliferation. Moreover, the Col/CS high scaffolds seeded with LNCaP cells showed the highest metabolic activity and proliferation throughout the 14 days cultures. The live/dead images showed only minimal dead cells. All Col/CS scaffolds seeded with PC-3 expressed cytokines associated with EMT processes, and Col/CS high had the highest signal intensity of ENA-78, IL-6, IL-8, Angiogenin, and MCP-1 cytokines among the scaffold types assessed.
Initial findings demonstrate the scaffolds can be used as an in vitro platform for studying PC

EMT processes and drug testing.

keywords: prostate cancer, scaffolds, biomaterial

62825424455

ON THE OPTIMISATION AND TAILORING OF THE ECM COMPLEXITY TO THE CANCER AND STROMAL CELLULAR COMPARTMENTS OF A BIOMATERIAL BASED NOVEL 3D MODEL OF PANCREATIC CANCER TISSUE

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INTRODUCTION:With a 5-year survival rate of only 11%,Pancreatic Ductal Adenocarcinoma (PDAC) is the 7th leading cause of cancer related death worldwide. The aggressive nature and high mortality rate of PDAC is partly attributed to the tumour microenvironment(TME) and its resistance to currently available treatment methods1.The TME consists of various structural, cellular and protein components. An in-depth study of PDAC biology and its resistance to current therapeutic methods requires the development of biomimetic, niche mimicking in vitro tumour models. We have previously developed a poly urethane (PU) based 3D pancreatic cancer model using (i) pancreatic cancer cells (monocellular model) and (ii) pancreatic cancer cells, stellate cells and endothelial cells, i.e., all of which are important elements of the pancreatic cancer cellular tissue microenvironment(multicellular model).We were able to show long term maintenance of the in vitro model (> 2 months), feasibility of extracellular matrix (ECM) mimicry, formation of dense cellular masses, secretion of ECM proteins,formation of realistic hypoxic gradients as well as effect of hypoxia2,3,4.
The current work focusses on further advancement of our PU scaffold assisted multicellular model5 via (i) the further optimisation of the ECM composition for the different cell compartments of our model (cancer vs stromal) (ii) the incorporation of immune cells in our model (the role of which is crucial for the disease development).

METHODS:PU scaffolds were prepared as per previously published protocols.Absorption based surface modification of the scaffolds enabled coating with ECM proteins (laminin, collagen I and fibronectin) for enhancement of ECM mimicry. Various cells (cancer, stellate, endothelial and immune) were incorporated in the model as previously described.The effect of different ECM proteins coatings and their combinations for the cancer compartment and the stromal compartment were systematically assessed long term (4 weeks). In situ assays (microscopy) and ex-situ(PCR) assays for monitoring the cell viability (for all cell types), spatial organisation, ECM and biomarker production were carried out at specific time points throughout the culture period.

RESULTS:We report a systematic comparative study to assess the effect of various ECM protein coatings for pancreatic cancer cells and stromal cells on PU scaffolds. Our study shows that cancer cell and stromal cells show preference for different ECM proteins and therefore tailoring the ECM composition (including ECM combinations) is essential for optimal/physiological in vitro

cell growth. Within the cancer compartment, different cell lines of pancreatic cancer showed differences in terms of cell growth, secreted ECM proteins and biomarker expression.

CONCLUSION:Our data show the importance of ECM proteins tailoring and combination for the growth, proliferation and cellular protein and biomarker secretion for cancer and stromal cells in vitro. Furthermore, our work highlights the importance of developing zonal/spatial cellular and matrix structures in 3D for the efficient mimicry of the pancreatic cancer tissue in vitro.

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keywords: pancreatic cancer, 3D in vitro model, scaffolds, tissue engineering

62825437555

SYNERGETIC EFFECT OF MECHANICAL STIMULATION AND ECM MICROENVIRONMENT ON LUNG FIBROBLASTS IN AN EX VIVO MODEL FOR IPF

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INTRODUCTION
Idiopathic pulmonary fibrosis (IPF) is a lung disease characterized by excessive remodelling of the extracellular matrix (ECM), where fibroblasts are one of the main producers of ECM components. Our aim is to understand how lung fibroblasts respond to mechanical cues in a healthy or diseased ECM composition. We have created an ex vivo model to mechanically stimulate fibroblasts repopulated in decellularized healthy or IPF lung tissue. We hypothesized that compositional ECM changes have an important impact on fibroblasts' translation of mechanical cues from its microenvironment thus influencing disease progression.

METHODS
IPF and healthy distal lung tissue were decellularized in slices (350 um) to produce acellular lung scaffolds. The scaffolds were attached to a custom-made device for cyclic stretch built in polydimethylsiloxane and repopulated with healthy primary distal lung fibroblasts. The scaffolds were either exposed to cyclic stretch (0.2 Hz, 10% strain) or cultured under static conditions up to 3 days and analyzed for cell viability, histology, RNA expression and released mediators.

RESULTS
The secretion of VEGF-A in the IPF conditions showed a ~50% increase compared to healthy conditions for both conditions at 24h. Interestingly, at 72h the secretion of VEGF-A in IPF scaffolds decreased under cyclic stretch compared to fibroblasts cultured in healthy scaffolds where secretion increased. The IPF scaffolds had a higher VEGF-A secretion statically compared to heathy scaffolds whereas it was lower than the healthy when stretched. When looking at gene expression patterns of CTGF and CYR-61, fibroblasts in IPF static scaffolds had a higher gene expression of CTGF than in healthy scaffolds at 24h. Interestingly, the opposite trend was observed in cells cultured under cyclic stretch. The impact of ECM and mechanical stimulation was significant (P=0.0351) in altering gene expression levels of CTGF.

DISCUSSION
Our results indicate that the translation of mechanical cues is dependent on structural and compositional ECM organization. Secretion of angiogenic factors (VEGF-A) and expression of mechanotransduction factors (CTGF and CYR61) became altered primarily through the impact of cyclic stretch or ECM, respectively. These findings indicate that there is a synergetic interaction between mechanical stimulation and ECM that impact cellular responses in chronic lung diseases.

keywords: ECM, cyclic stretch, mechanotransduction, lung, fibroblasts

52354519305

SYNTHETIC PRE-VASCULARIZED POROUS SCAFFOLD AS AN ENGINEERED ENVIRONMENT FOR THE IN VITRO OSTEOSARCOMA MODEL

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One of the biggest challenges in the field of tumour engineering is providing cells with a proper environment mimicking physiological conditions of cancer from the biomechanical point of view. Hard tissue cancers, such as osteosarcoma, are no exception, especially taking into account the complexity of the bone microenvironment. The golden standard is the use of human-derived tissue-engineered scaffolds, but this meets other difficulties connected to regulations, low availability and reproducibility. This work aims to find an approach for providing osteosarcoma cell spheroids with the physiological environment independently of human tissue availability.
To form stable cell aggregates, GFP-transfected human osteosarcoma cells (U2OS) were cultured in a multiwell agarose-coated plate. The dynamics of cell metabolic activity in spheroids and their morphology was monitored by resazurin reduction assay, as well as visually by light and fluorescent microscopy. Alginate and bioactive glass were the components of choice for producing the matrices due to their proven reputation in the biomaterials field. Polymerized alginate samples enriched with bioactive glass particles were frozen, freeze-dried, cut and analysed by scanning electron microscopy (SEM). Human endothelial cells (EA.hy926) were suspended in the collagen and seeded on the porous alginate-based matrices. The cell-loaded scaffolds were then cultured with the addition of vascular endothelial growth factor (VEGF) to trigger tubule-like network formation. After the network development was confirmed by fluorescent microscopy and histological assays, previously obtained osteosarcoma spheroids were placed within each scaffold and monitored during the cultivation period to assess metabolic activity and sprouting.
Throughout the set-up of the method and considering the data from the analyses above, the optimal parameters of preparation of the matrices were established. Endothelial cells seeded on the porous matrices showed sufficient viability rates and the ability to form a tubule-like network throughout the chosen periods. Also, osteosarcoma cell spheroids demonstrated high metabolic activity and tumour-like behaviour in terms of invasion the surrounding matrix when placed within the pre-vascularized scaffolds.
The viability and morphology of endothelial cells and, consequently, osteosarcoma spheroids, allowed us to conclude that the obtained alginate-based pre-vascularized porous scaffolds are suitable as an environment for 3D in vitro modelling of osteosarcoma. The architecture of the scaffolds resembles trabecular bone tissue, while the inclusion of bioactive glass particles allows avoiding biochemical activation of osteogenic conditions. Moreover, the proposed system is tuneable, which offers the possibility of introducing various cell lines to simulate osteoblast-osteoclast crosstalk, as well as interaction with immune cells, and setting up dynamic conditions. The described approach can be admitted as promising in terms of developing antitumor drug test systems and studying peculiarities of primary bone cancer.

keywords: osteosarcoma, tumour engineering, pre-vascularized scaffold

94238188569

TISSUE ENGINEERING RESOURCE CENTER – TRAINING, DISSEMINATION AND OUTREACH FOR THE NEXT GENERATION OF TISSUE ENGINEERS

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Introduction: The Tissue Engineering Resource Center (TERC) was established to serve the established, junior and aspiring investigators and trainees by providing advanced technologies, training and service; as well as students and the general public by providing dissemination and outreach programs. Our ambition is to offer resources that will be transformative to the overall impact of tissue engineering on science and its translation.

Methodology: We sought to engage bioengineers across career levels and democratize the accessibility of "hands on" tissue engineering tools, and mentorship by offering remotely a portfolio of new resources. As a part of training, we created the Tissue Engineering Career Conversations podcast envisioned to shed light on a wide range of career trajectories, academic and non-academic, that have emerged from the bioengineering community. As a part of dissemination, we host the weekly Tissue Talks seminar series. As a service to the field, we held the Next Generation Tissue Engineering Symposium with the current and emerging leaders providing perspectives on progress in our field. As a part of outreach, we created the Science Simplified series, exploring cutting edge scientific publications in the tissue engineering field in a way suitable for future bioengineers among K-12 students.

Results: Through our website nextgenterc.com and social media platforms, we are interacting with a broad international community in our field, and creating resources that are accessible remotely from anywhere in the world, free of charge. As a result, the Next Generation Tissue Engineering Symposium gathered participants from all continents (Fig 1).

Conclusion: The goal of the TERC is to promote tissue engineering and establish a community for continued exchange of ideas and collaborations among emerging bioengineers. To this end, our group is continuously developing new approaches for training, dissemination and outreach. TERC is providing a highly effective and unique environment, a two way street for our trainees to lead the dissemination and outreach activities, and to learn and practice their utilization.
Acknowledgements We gratefully acknowledge NIH funding support of P41EB027062.

keywords: tissue-engineering, bioreactors, outreach, dissemination, training

PS03
3D printing of bionic organs – how far are we from clinical application?

20941839055

BIONIC PANCREAS – 3D BIOPRINTING OF A BIONIC ORGAN WITH A VASCULAR SYSTEM – RESULTS OF TRANSPLANTATION IN LARGE ANIMALS.

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There is lack organs for transplantation. The transplantation of the pancreas is recommended in diabetes with complications. The combination of cell biology and 3D-bioprinting can create organs with vasculature which should be functional. There are some issues to be solved: leakage, thrombosis, enhancement against high pressure, connecting organ to the recipient. The purpose of this study was to 3D-bioprint bionic pancreas which maintain a stable flow in large animals. Material and methods: 3 pancreas geometries were calculated and bio-printed. The mechanical properties were tested in a bioreactor-20 tests. After bioprinting, the external vessels were attached: decellularized vessels(DV) and vascular prostheses(VP). Studies in animals were carried out on 5 pigs. Results: Bionic pancreas with internal vessels of 1 mm was bio-printed. Bionic pancreas were stable up to 200 mmHg of pressure in bioreactor. In animals there was a flow throughout the organ after release of vascular clamp. In two cases(DV)-sudden stop of flow within one hour from transplantation with cloths in DV was observed. In next three cases VP were used. In third case stable flow through the organ was observed for 24 hours. Then a thrombotic cloth was observed within an organ. Cloths were in the intersection between printing lines of the internal vessels which were printed transversely to the flow. The g-code and model were changed. Then stable flow was observed with ultrasound and radiographs examination during observed period in following cases. Conclusion: 3D-bioprinted organ can be successfully fixed to the recipient and maintain flow through the organ.

keywords: pancreas, vascular connection, bioprinting, bionic organs

20941876059

MATURATION AND EVALUATION OF 3D PRINTED BIONIC PANCREAS WITH A DEDICATED BIOREACTOR.

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The technology of 3D printing of bionic organs gives an opportunity to solve the problems of classical transplantology, such as the shortage of organs, complications of immunosuppression or rejection. After printing, the bionic pancreas requires an optimal environment conditioning the process of its maturation, which consists in the colonization of the produced vessels with endothelial cells and the tubularization of the endothelium within the microcirculation. After the maturation process is completed, it is necessary to evaluate the resulting organ in terms of its functionality and safety.
In order to minimize the risk of contamination while ensuring the necessary functionalities, a bioreactor was created that allows the maturation of the bionic pancreas, and after the end of the process, functional assessment using the semi-automatic Glucose-Stimulated Insulin Secretion (GSIS) test and the assessment of vascular tightness using the pressure test.
10 procedures of maturation and bionic pancreas evaluation were performed using a bioreactor. 5 pancreas contained human beta cells (450000000 cells) and 5 pancreas contained porcian isolated pancreatic islets (75,000 IEq). The mean pancreatic maturation time was 23 hours (2-72 hours). The effectiveness of adhesion of endothelial cells to the vascular wall and tubularization of endothelial cells was assessed by immunohistochemistry. The GSIS test was performed by automatically replacing the medium with glucose at various concentrations. The integrity of the vascular system was assessed by maintaining a pressure of 190 mmHg for 5 minutes.
The adhesion of endothelial cells to the bioink was observed after 1 hour. Tubularization of the endothelium was observed after 48 hours. Insulin secretion upon stimulation with glucose was observed without delay to the control (beta cells or islets) and the insulin concentration during the observation showed a constant ratio compared to the control, but without a clear peak at high glucose concentration. In 8 out of 10 pancreas, no vascular leakage was observed during the pressure test. No material contamination was observed during pancreatic perfusion.
The use of a dedicated bioreactor enables safety during the bionic maturation process of the organ, while allowing for an effective assessment of the organ's functionality and the tightness of the vascular system.

keywords: pancreas, bionic organ maturation, bioprinting, bionic organs

PS05
Additive manufacturing in tissue repair: current status and obstacles toward a daily clinical practice

31412741587

DESIGN, MODELLING & BIOFABRICATION OF INTERFACE-FREE OSTEOCHONDRAL DEFECT REPAIR

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An Osteochondral defect (OCD) refers to an area of local damage in the articular cartilage and in the underlying subchondral bone (SB). Trauma related to an injury, or a long-term chronic disease are the main causes of this defect. Tissue engineering osteochondral scaffolds (OCSs) is a method used for the treatment of OCD. This method could achieve the regeneration of the damaged tissue using scaffolds and cells. One key research gap is to solve the complexity of manufacturing a multiphasic graded OCS, involving different layers of articular cartilage, cartilage-bone interface, and subchondral bone with no interface which can avoid the delamination between layers, helping to achieve the specific mechanical properties of these tissues. The manufacturing process used is 3D printing extrusion using the 3D discovery printer (RegenHU) which allows multi-material deposition, in this project two different materials are being used for printing different phases in a single step: gelatin methacrylate (GelMA), and polycaprolactone (PCL). These materials are selected as the best candidates for the biofabrication of OCSs, which are evaluated in terms of swelling and degradation. One of the most promising approaches relies on complex design to fulfil the mechanical properties of the osteochondral tissue, such as stress and strain, validated by finite element analysis (FEA) of the scaffold design. The Young's modulus of the PCL/GelMA scaffold verged to the osteochondral native tissue. Although these results demonstrate the potential of the design related with the mechanical properties of the OCS, improvements require more complex design and future studies will focus on their optimisation, alongside further mechanical, swelling and degradation tests of the scaffold and cell culture evaluations of the OCS.

keywords: Osteochondral, 3D printing, gelatin methacrylate, and polycaprolactone

83767224157

THREE-DIMENSIONAL CANCER MODEL IN THE LAB: A TOOL TO ADVANCE DETECTION AND THERAPY OF HIGH-GRADE BRAIN CANCER

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Introduction:
Petridish-based 2-dimensional (2D) cell culture models are currently used as the first step to test the efficacy of anticancer drugs, but they don't accurately mimic tumor biology and microenvironment. Moving to 3D models, one of the major concerns is how to assess the drug efficacy. Cell viability is an essential parameter to be monitored to measure cell growth and to assess the effectiveness of a drug candidate in cancer research. Several cell viability assays are commonly used but these assays are typically used in low cell density 2D cultures and may not produce the same results in 3D cultures with high cell density and added matrix components. Our aim is to develop a realistic preclinical 3D brain tumour model from native tissue and evaluate the accuracy of our 3D model to reflect a realistic drug response.

Methodology:
3D brain tumour model were developed by decellularizing animal brain tissue followed by seeding with U251 cells. Identical U251 cell numbers were plated in 2D and were mixed with Matrigel (a common 3D cell culture platform) to form a 3D culture model. We compared the results of three different viability assays (MTT, Alamar Blue, ATP) on three different culture models and calibrated the assay output versus known cell numbers by counting and DNA quantification.

Results:
In all of our selected cell viability assays, the assay output was higher in 2D samples compared to matrigel samples containing the same number of cells. In our novel 3D brain tumour model, ATP assay and Alamar Blue assay showed the most similarity to the DNA assay. MTT showed the most difference in assay read outs in 2D vs Matrigel, the same result reflected in the scaffolds.

Conclusion:
The results suggested the 3D brain tumour model performed better than Matrigel based-model in terms of accuracy of assay readouts (ATP and Alamar Blue). MTT was observed to not measure the viability of the cancer cells accurately for all 3D models. Not all protocols for viability assays that are validated for 2D cell culture are directly applicable to 3D models. Further optimization and calibration are required for 3D cell culture.

keywords: 3D cell culture, Extracellular matrix, Glioblastoma, Cell viability assay

83767203255

VERSATILE MULTI-CROSSLINKING PHENOL MODIFIED ALGINATE AS (BIO)INK PLATFORM FOR BIOPRINTING

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INTRODUCTION
Bioprinting is gaining an ever-increasing research interest thanks to its promising new applications in the field of tissue engineering. The main issue regarding this biofabrication technique is the engineering of suitable bioinks, accommodating both the requirements for fabrication and for cell culture and signalling. Versatile platform bioinks, with easily tailorable rheological, mechanical and biological properties could be applied for various applications and represent a powerful tool to overcome issues related with the long process required to engineer and validate inks. A versatile platform based on tyramine modified alginate (AL-TY) with multiple available crosslinking routes was defined, with the possibility to introduce tissue-specific biological functionalizations with phenol moieties during crosslinking.

METHODOLOGY
Alginate was functionalized with tyramine through carbodiimide chemistry as already reported [1], obtaining a degree of substitution of 36 ± 2 % (characterized by 1H.NMR). Various crosslinking methods were tested and AL-TY was found suitable for: ionic gelation (Ca^{2+}), enzymatic gelation (Horseradish peroxidase), photo-crosslinking (riboflavin (Rb) as photosensitizer and sodium persulfate as an electron acceptor). A novel crosslinking photo-enzymatic crosslinking route was investigated, as well, by activating the enzyme with light and Rb. The crosslinking kinetics of the various methods alone and combined were analysed through rheology and photo-rheology. The effect of crosslinking parameters (i.e. concentration of various reagents, light exposure time, light source power, etc.) was assessed to identify the best way to optimize the crosslinking kinetics and the mechanical properties of the constructs. Given the bioinert nature of alginate, tripeptide Arg-Gly-Asp (RGD) was introduced to favour cell adhesion. Cyclo(RGDyk) was selected, as containing a phenol group, it can be introduced during crosslinking [2]. The RGD concentration effect was evaluated on encapsulated human bone marrow-derived mesenchymal stem cells (hMSC). As a proof of concept, deferoxamine (DFO) was grafted onto AL-TY to validate the possibility of introducing additional biological cues in the bioink. DFO has been reported to behave as a hypoxia simulator, promoting the expression of genes associated with angiogenesis [3]. The platform was finally validated for bioprinting, by fabricating constructs under different conditions and measuring their mechanical properties, swelling and degradation time.

RESULTS AND CONCLUSIONS
All tested crosslinking routes proved to be suitable to produce hydrogels. However,

photocrosslinking alone produced hydrogels with extremely fast degradation times, due to damage of the polymeric chains caused by excessive radical production. The extensive rheological characterization of the crosslinking methods allowed to verify the effect of several process parameters on the reaction kinetics and yield, and to build preliminary guidelines to tailor the properties of the gels and effectively create a versatile platform system in terms of mechanical properties and crosslinking routes and kinetics. Similarly, the addition of cyclo(RGDyk) and the effect of its concentration on cell adhesion was tested by preliminary cell encapsulation experiments. In conclusion, the material is versatile and can be easily tailored in terms of rheological properties, final mechanical properties, crosslinking kinetics and required biological properties. Additionally, the material is suitable for bioprinting and is showing promising results in building a "universal" bioink.

keywords: Multi-crosslinking platform, Alginate-tyramine, Bioprinting, photo-enzymatic crosslinking

PS06
Advanced Biotechnology and Biofabrication approaches for soft tissue engineering and in vitro models: the ENLIGHT and BIRDIE perspective

73296336405

DESIGN AND ADDITIVE MANUFACTURING OF SCAFFOLDS FOR LARGE-VOLUME SOFT TISSUE ENGINEERING

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Introduction: Soft tissue-engineered constructs (sTECs) require tailored dynamic changes in their structure-properties-process (SPP) relations to preserve their functionality throughout the tissue regenerative process. At the initial stages of regeneration, a highly interconnected architecture is recommended to accelerate plaque adhesion and blood clot formation. At later stages of regeneration, a dense lattice structure is not entirely needed whereas a mechanically supportive structure with a greater extent of free space for newly formed tissue is recommended to avoid bridging and capsular contraction. Mechanical stability of sTECs, which plays a critical role in minimizing load transfer to new tissue, should remain until subsequent remodelling stages occur. Otherwise, at the cellular level, excessive stress and strain inhibit cell proliferation and differentiation, and at the tissue level, the lack of a physical substrate to withstand external loads leads to incomplete, inadequate and compromised large volume regeneration. Another positive impact of long-term mechanical integrity is enhancing shape retention of the structure and facilitating tissue formation towards complex anatomical shapes, especially in a large scale where larger tractional forces exist. Lack of attention to these SPP relations is one of the underlying reasons for the failure of sTECs in large tissue volumes where complex inter-scale relations between mechanics, biology and topology exist. This study aims to establish an adaptable workflow from materials to design, optimization, and manufacturing to tailor and customize structural/mechanical properties and behaviours for sTECs under different loading conditions.

Methods: Based on well-understood properties of new medical-grade polymers, novel rational design strategies were established to modulate the local degradation profile of composite structures and address required SPP relations essential for sustained large-volume soft tissue regeneration. Programming-assisted dual printing was utilized to deposit different materials in pre-designed patterns and go beyond bulk properties of single-material structures. Comprehensive in vitro and in vivo analyses were performed on non-degraded and degraded composites.

Results: The design-dependent workflow allowed to locally tune the degradation profile of

the composites and address long-term mechanical stability and shape retention to minimize excessive external loads applied to newly formed tissue at early as well as later stages of regeneration. It also allowed modulating an increase in mechanical conformity and free volume expansion which led to a higher compliant deformation as well as larger free spaces for growing tissue. The other highlighted outcome of this platform was a controllable tissue guidance pathway and subsequently, organized tissue and blood vessels formation within the expanded pores which appears to support later stages of tissue formation and angiogenesis.

Conclusion: Multi-material printing of medical-grade polymers combined with rational design strategies and well-understood material properties provides a transplantable customization pathway to develop multi-functional scaffolds with tailored structural-mechanical properties which address multifaceted challenges associated with large-volume soft tissue regeneration. The proposed methodologies open new avenues towards sustained large-volume soft tissue regeneration.

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keywords: Soft tissue regeneration, Composite 3D printing, Structure-properties-process relation, Large volume scaffolds

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ESSENTIAL STRUCTURE-PROPERTIES-PROCESS RELATIONS FOR SUSTAINED LARGE-VOLUME SOFT TISSUE REGENERATION

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Introduction: Soft tissue-engineered constructs (sTECs) require tailored dynamic changes in their structure-properties-process (SPP) relations to preserve their functionality throughout the tissue regenerative process. At the initial stages of regeneration, a highly interconnected architecture is recommended to accelerate plaque adhesion and blood clot formation. At later stages of regeneration, a dense lattice structure is not entirely needed whereas a mechanically supportive structure with a greater extent of free space for newly formed tissue is recommended to avoid bridging and capsular contraction. Mechanical stability of sTECs, which plays a critical role in minimizing load transfer to new tissue, should remain until subsequent remodelling stages occur. Otherwise, at the cellular level, excessive stress and strain inhibit cell proliferation and differentiation, and at the tissue level, the lack of a physical substrate to withstand external loads leads to incomplete, inadequate and compromised large volume regeneration. Another positive impact of long-term mechanical integrity is enhancing shape retention of the structure and facilitating tissue formation towards complex anatomical shapes, especially in a large scale where larger tractional forces exist. Lack of attention to these SPP relations is one of the underlying reasons for failure of sTECs in large tissue volumes where complex inter-scale relations between mechanics, biology and topology exist. This study aims to establish a transplantable workflow from materials to design, optimization, and manufacturing in an effort to tailor and customize structural/mechanical properties and behaviours for sTECs under different loading conditions.

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Results: The design-dependent workflow allowed to locally tune the degradation profile of the composites and address long-term mechanical stability and shape retention to minimize excessive external loads applied to newly formed tissue at early as well as later stages of regeneration. It also allowed modulating an increase in mechanical compliance and free volume expansion which respectively led to a higher compliant deformation as well as larger free spaces for growing tissue. The other highlighted outcome of this platform was a controllable tissue guidance pathway and subsequently, organized tissue and blood vessels formation within the expanded pores which appears to support later stages of tissue formation and angiogenesis.
Conclusion: Multi-material printing of medical-grade polymers combined with rational design strategies and well-understood material properties provides a transplantable customization pathway to develop multi-functional scaffolds with tailored structural-mechanical properties which address multifaceted challenges associated with large-volume soft tissue regeneration. The proposed methodologies open new windows towards sustained large-volume soft tissue regeneration.

keywords: SPP relations in Soft tissue, New design and bio-fabrication approaches in soft tissue, Additive Manufacturing and

toolpath planning, Guided tissue formation in large-scale

20941851037

FLEXIBLE AND TOUGH 3D PRINTED MESHES FOR SOFT TISSUE RECONSTRUCTION

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Simultaneous flexibility, strength and toughness are exceptional characteristics of soft biological tissues which do not exist in man-made materials. Soft tissue-engineered meshes (sTEMs) should exhibit tissue-like mechanics and geometries for better performance and fit. However, the majority of current clinical meshes for soft tissue regeneration are conventional fabrics made by knitting or weaving, which have limited fabrication capabilities to adapt the meshes with diverse mechanics and geometries. This limitation has led to noncompatible conformability and compliance, which result in undesired mesh performance and fit. Moreover, the lack of local reinforcement of regions that require stronger supports has increased the risk of mechanical failure of the meshes as they shrink. GalaformTM is only commercialized sTEMs with a reinforcing rim for plastic and reconstructive surgery; however, it does not offer customized meshes with patients' specific reinforcements. In this study, we established a biomimetic design strategy and a manufacturing platform to develop flexible, strong, and tough meshes for soft tissue reconstruction.
A biomimetic design strategy was developed to control the non-linear mechanical response of meshes at a unit-cell scale and incorporate a stretchable polymer, Strataprene® (ST), and a strong polymer, Caproprene® (CAP) or Lactoprene® (LAC) in a composite fashion to simultaneously address the required flexibility, strength, and toughness within one integrated structure. The design is composed of two series of sinusoidal curves with tailored amplitudes to control the level of polymers' contributions at three different stages of deformation. On the one hand, the smaller curves are attributed to the stretchable polymer since they straighten faster than large curves and should tolerate a high elongation to allow further stages of deformations to occur. On the other hand, the larger curves are attributed to the stronger polymers to support a rapid increase in the strength of the structure as they are lined up at high load regimes.
Utilizing multi-material printing offered by FFF technique and toolpath planning programmed by MATLAB scripts, these polymers were placed in pre-designed patterns. Two series of meshes with uniaxial and biaxial strength were fabricated and characterized comprehensively.
Under tension, the meshes indicated a three-stage mechanical response, where at stage 1, ST curves turned into straightened position and induced the first dynamic transition in the architecture leading to a J-shape stress-strain response. At stage 2, CAP or LAC curves were aligned parallelly to the deformation direction and produced the second J shape response with a higher level of stress compared to stage 1. At stage 3, both polymers were fully elongated and exhibited a linear elastic response. Since CAP and LAC have a significantly higher elastic modulus when compared to ST, they mainly dominated the mechanical response of the last stage and increased the strength of the structure rapidly. Therefore, the structure highly indicated stretchability at low load regime while tolerating large stress and absorbing high energy at high load regime.
The proposed platform offers a high level of design flexibility to develop both mechanically and geometrically functional and customized scaffolds, not achievable by the currently commercialized meshes in the market.

keywords: Additive manufacturing, Soft yet tough meshes, Bio-Inspired design

73296341466

HOW TO DERIVE STABLE ACOUSTIC DROPLET EJECTION PARAMETERS FOR COMBINED MACRO AND MICRO LEVEL BIOPRINTING

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During the last decade, bioprinting technology has gradually made tremendous progress in manufacturing complex cell-laden hydrogel-based constructs especially for advanced 3D in vitro models. However, most models still lack full complexity. The combination of multiple state-of-the-art printing processes is currently expected to make up for this shortcoming and to improve the outcome after cultivation.
Most state-of-the-art bioprinting processes typically use a nozzle to print droplets or extrude material. The nozzles are a critical component because they must be selected with a tiny diameter for high-resolution printing and quickly become clogged during the printing process. As the nozzle diameter is reduced and the speed in the nozzle is increased, the shear stress increases. Critical shear stresses lead to sustained damage of the printed cells. The acoustic droplet ejection process presented here does not require a nozzle at all. Therefore, nozzle-related wall shear stress is eliminated. The technique, which has so far been associated mainly with single-cell printing1, has recently been made usable for macroscopic 3D bioprinting2. However, this printing method is new to the field of biofabrication and has not yet been fully characterized.
Individual droplets were generated by an ultrasonic burst that applied sufficient energy below the liquid surface to overcome the surface tension and inertial forces of the bioink against gravity. Droplets ranging from picoliters to nanoliters were generated. By simple adjustment of the ultrasound, droplets of the order of a single cell can be printed on the one hand, or large droplets of the order of cell agglomerates on the other. The generated cell-laden hydrogel droplets attach gently to a building platform. This way, 3D constructs of several millimeters in size were successfully realized by moving the building platform. The ejected droplets were captured in-flight with a 5-megapixel camera (VCXU-51M, Baumer) and microscope lens. A controlled LED flash captured the droplets during the exposure time. Contour analysis was performed using TwinCAT Vision (Beckhoff). For the evaluation of printable bioinks, Pluronic F-127 and Matrigel were characterized with the camera system. The fluorescent dye CellTracker (Invitrogen, Thermo Fisher Scientific) was used to monitor the location of cells within the 3D-printed construct.
Using the camera approach, it was possible to reveal optimal ultrasound parameters. A characteristic phase map was created for each tested bioink. From these phase maps with their different regimes, the best parameter combinations can be derived for each bioink.
3D structures were printed at best parameter combinations and inspected using confocal microscopy, respectively.
These results are of importance for material-specific optimal bioprinting using acoustic droplet ejection principle. Using the described phase map method, novel hydrogel materials can be quickly evaluated with regard to optimal ejection parameters. This enables optimal acoustic printing and helps to increase the complexity of 3D printed structures. Thus, more precise spacial placement of cell-laden hydrogel droplets at higher printing resolution will be feasible in the future using the acoustic bioprinting technique.
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2. Jentsch, S. et al., Small Methods, 5, 2000971 (2021).

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keywords: 3D bioprinting, multiscale, acoustic droplet ejection

83767236279

RETINA BIOFABRICATION USING NOVEL ELECTROSPUN NANOFIBROUS SCAFFOLDS AS PROSTHETIC BRUCH'S MEMBRANE

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Introduction: Age-related macular degeneration (AMD) is a major cause of blindness worldwide, affecting 170 million people1. AMD consists in a degeneration of the central retina due to age-related changes in Bruch's membrane (BM) and in the retinal pigment epithelium (RPE). Currently, periodic intravitreal injections of anti-vascular endothelial growth factor drugs are the gold standard therapy in the management of AMD. However, these drugs are unable to restore tissue functionality. Retinal tissue engineering may be helpful in providing better solutions. This work aims at designing a functional BM.

Methodology: We fabricated electrospun nanofibrous membranes composed of Bombyx mori silk fibroin (BMSF) and polycaprolactone (PCL). We investigated both aligned and randomly oriented nanofibrous mats to study the influence of fibre orientation on tissue regeneration. We evaluated the following scaffold properties: fibre morphology, thickness, permeability, mechanical properties, in vitro cytocompatibility, and the formation of mature epithelial monolayers. We employed ARPE-19 cells and zonula occludens (ZO)-1 staining for in vitro studies.

Results: BMSF/PCL membranes with aligned and random fibres were successfully produced by electrospinning. The thickness of aligned and randomly oriented fibrous mats was 58.6 µm and 40 µm, respectively. The randomly oriented fibrous membrane displayed an average fibre diameter of 938±223 nm, whereas the aligned fibres showed a slightly higher average diameter. Mechanical properties were investigated as they are crucial for BM structural role and surgical applications. The elastic modulus of the aligned and randomly oriented fibrous mats was 43.4±9.1 and 13±3.6 MPa, respectively. Transport is another important function of native BM; therefore, membrane hydraulic conductivity (Lp) was assessed. We found a Lp of 2.35×10-8 and 20x10-10 m/(Pa*s) for the aligned and randomly oriented fibrous membranes, respectively. Moreover, we proved the biocompatibility of the mats and the formation of a functional epithelial monolayer. The expression of ZO-1 on the aligned fibrous membrane presented a higher staining intensity than on the randomly oriented fibrous scaffolds suggesting that the fibre orientation promotes a proper cell organization.

Conclusion: An ideal substrate for constructing a prosthetic BM with attached RPE cells has yet to be found. Both the obtained mats showed similar properties to human BM, which has a fibrillar network, a elastic modulus ranging from 6 to 14 MPa, and a hydraulic conductivity ranging from 20 to 100 x10-10 m/(Pa*s)2. Currently, we are carrying out additional studies to confirm that oriented fibres enhance biological tissue regeneration. The successful outcome of this study will inform the treatment of an optimal substrate for RPE transplantation.

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2. Chan, W.H., et al. IOVS, 48, 2187 (2007).

keywords: retina tissue engineering, ophthalmology, electrospinning, age-related macular degeneration

62825432139

TOWARDS PHYSIOLOGICALLY RELEVANT BIOPRINTED KIDNEY IN VITRO MODELS

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Chronic kidney diseases (CKD) is the twelfth leading cause of death worldwide, where patients present a gradual loss/impairment in kidney function, leading to end-stage renal disease (ESD). People suffering from ESD have limited life-saving temporal renal replacements options, such as the kidney dialysis, while donor organ transplantation is still the current gold standard, generally hampered by the limited organ availability. The need of replacement therapies is being investigated worldwide. In vitro platforms to study CKD might facilitate further understanding of its progression and eventually the development of new therapies. Biofabrication approaches such as 3D printing and bioprinting offer the possibility to produce pre-designed in vitro models and potentially new therapies. Several approaches are currently under investigation, such as scaffold, stem cell based therapy and decellularised animal tissue, but these are far from being alternatives. Therefore, reliable in vitro models are clearly needed. We developed extrusion based bioprinted chip models which present multiple advantages compared to the classical microfluidic chips, in terms of reproducibility, cost, production time and feasibility of varying geometry. We used bioprinting to produce sacrificial pluronic filament structures that were subsequently encapsulated in PDMS. The pluronic was then removed, creating empty channels that were subsequently seeded with cells. Primary renal epithelial cells (keratin 8-yellow fluorescent protein, Krt8-YFP) and human umbilical vein endothelial cell (HUVEC) were used. Results showed that it was possible to bioprint channels in varying diameters. The effect of channel geometry, its reproducibility and coatings for optimal cell culture were all evaluated. Cultured Krt8-YFP and HUVEC cells adhered and proliferated until a complete lumen was formed. Our model presents single and double channels, demonstrating the capability to mimic not only the renal tubule, but also the capillary side, together with extra-cellular matrix (ECM) hydrogels dispensed in the middle of the two channel, mimicking in vitro the renal tubulointerstitium. In conclusion, we believe this tubulointerstitium model can be a first step toward the production of more complex disease models for testing new therapies for CKD.

keywords: Kidney, bioprinting, biofabrication, disease, in vitro model

PS07
Advances in cardiac tissue engineering: in vitro platforms and in vivo regeneration

94238145005

BIOFABRICATION OF MINIATURISED, PATIENT-SPECIFIC HYDROGEL VESSELS

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Globally, approximately thirty-one percent of worldwide deaths are caused by cardiovascular diseases (CVD). Most CVDs are usually a late-stage clinical manifestation of atherosclerosis, which has been shown to be dependent on the complex interactions between various physical and biological factors of a blood vessel in a patient-specific manner. These factors include the anatomy of the arteries, which dictate haemodynamic flows that interact with the vessel endothelium to develop inflammation and lipid accumulation. Vascular models are widely used to study atherosclerosis and test the efficacy of CVD treatment modalities. However, current vascular models developed to date can only either recapitulate the physical (i.e., geometry and haemodynamic) or biological (i.e., endothelium) environments of an artery. Therefore, there is a disconnection in the understanding of how physical factors interrelate to the biological responses of a blood vessel.

The present study aims to combine additive manufacturing and microfluidic organs-on-chip technologies to create a patient-specific vessel model that is capable of supporting the perfusion culture of vascular cells. This was achieved by reconstructing patient-specific arterial anatomies from phase-contrast MRI images and scaling down both anatomical geometries and haemodynamic information to a 1:4 model in order to facilitate perfused cell cultures. Next, a hydrogel vessel replica was fabricated by 3D printing a Poly (ethylene glycol) diacrylate (PEGDA) mould that was later impregnated with CaCl2 to trigger the self-assembly of alginate prepolymer which was perfused through the mould. The resultant hydrogel vessel constructs replicated specific vessel geometry and offer higher biocompatibility for cell culture than normal 3D printable materials. By integrating these structures with a physiological relevant flow, a vascular model that intends to include the homeostatic factors can be developed.

The work has not been published yet and it is open to changes in methods since it is under experimental fase.

keywords: Biofabrication, in-vitro, hydrogel, vascular

94238127724

BIOMIMETIC SCAFFOLD-BASED IN VITRO PLATFORMS RESEMBLING THE MAIN FEATURES OF HUMAN MYOCARDIAL FIBROTIC TISSUE

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Introduction: Cardiac fibrosis is a common pathophysiologic mechanism of most myocardial diseases like myocardial infarction (MI), associated with impaired cardiac function and arrhythmias, preventing the heart from effectively satisfying the metabolic demand of the body[1]. Following cardiomyocytes injury, adverse fibrotic remodeling results from the altered activity of cardiac fibroblasts (CFs), which upon mechanical or biochemical stimuli (e.g., TGF-β profibrotic cytokine) undergo phenotypic changes toward fully differentiated myofibroblasts (MyoFs)[2]. MyoFs expresses high levels of contractile proteins such as alpha-smooth muscle actin and they markedly enhance the deposition of ECM components like fibronectin and collagens. The increase in ECM proteins amount is accompanied by progressive architecture disruption causing anisotropy loss. Due to these structural changes, myocardial stiffness increases from few kPa to 35-70 kPa. Heart transplantation remains the only regenerative medicine approach in case of severe cardiac fibrosis. For this reason, reversing cardiac fibrosis is still a key challenge for cardiac regenerative medicine research. In the context of the preclinical validation of new advanced therapies/drugs, the use of highly reliable human in vitro models could be useful to overcome the limitations of traditional cultures and animal in vivo models low predictability and ethical controversies. In this work, we propose the development of a biomimetic in vitro human model in which both morphological and biochemical cues are integrated to mimic specific native microenvironment features for drug testing purposes.

Methodology: Polycaprolactone (PCL) based scaffolds with random morphology nanostructure were fabricated by solution electrospinning technique in order to provide topographical and mechanical cues. Electrospun PCL scaffolds were surface grafted with human collagen type I and fibronectin (PCL/polyDOPA/C1F), exploiting a mussel inspired approach, in order to mimic the ECM protein microenvironment of fibrotic tissue and support human cardiac fibroblasts (HCFs) culture, which mainly populate native cardiac fibrosis. Lastly, biochemical profibrotic stimulus was integrated by TGF-β addition to culture medium in order to study fibroblasts activation under different culture conditions. Each step of modeling was thoroughly characterized by morphological (SEM, immunofluorescence), mechanical (wet and dry AFM tests), chemical (BCA colorimetric assay), physical (QCM-D) and biological analyses (viability/cytotoxicity assay, immunostaining, activation markers quantification).

Results: Morphological characterization demonstrated the collection of PCL scaffolds with homogeneous fibers and pores size distributions. Immunostaining for collagen type I and fibronectin coating on PCL membranes, demonstrated successful proteins grafting mimicking the main features of human fibrotic ECM. BCA assay confirmed biomimetic coating deposition and its stability during 7 days test. AFM mechanical characterization demonstrated that PCL/polyDOPA/C1F scaffolds, in wet conditions, resemble fibrotic tissue stiffness behavior. Biological validation showed that PCL/polyDOPA/C1F scaffolds support HCFs adhesion, proliferation and activation even without TGF-β biochemical stimulus.

Conclusions: In conclusion, this platform recapitulates fibrotic tissue random structure, stiffness

and ECM composition. The proposed in vitro model of human cardiac fibrosis could be a promising biomimetic platform for preclinical testing of new advanced regenerative therapies thanks to its ability to replicate fibrosis hallmarks.

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keywords: Cardiac fibrosis, ECM remodeling, scaffolds, in vitro models

62825457368

BIOPATTERNING OF 3D CELLULAR STRUCTURES VIA CONTACTLESS MAGNETIC MANIPULATION FOR DRUG SCREENING

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Patterning and manipulation techniques have been used to fabricate 3D cell cultures in tissue engineering. The contactless magnetic manipulation approach is a rapid, simple, and cost-effective method that requires paramagnetic agents [1-3] or magnetic materials [4]. Here, to obtain patterned 3D cellular structures a new alginate-based bio-ink formulation was developed to fabricate 3D cellular structures using contactless magnetic manipulation. 3D cardiac model was obtained by patterning rat cardiomyocytes. Cellular and extracellular components and cardiac-specific markers of patterned 3D cellular structures were indicated successfully. Drug response of patterned 3D cellular structures was evaluated by applying doxorubicin. Patterned 3D cardiac cellular structures showed significantly different drug response compared to conventional 2D cell cultures. In conclusion, this technique provides an easy, efficient, and low-cost methodology to fabricate 3D cardiac structures for drug screening.

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keywords: contactless magnetic manipulation, 3D cell culture, drug screening

83767237746

DESIGN OF THREE-DIMENSIONAL BIOARTIFICIAL STRETCHABLE SCAFFOLDS THROUGH ADDITIVE MANUFACTURING FOR AN IN VITRO MODEL OF FIBROTIC CARDIAC TISSUE.

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Introduction: Cardiac fibrosis arises after myocardial infarction, causing progressive heart failure. New regenerative medicine approaches are under investigation to reduce or revert cardiac fibrosis.
In agreement with the 3Rs principle, testing in in vitro platforms mimicking pathological cardiac tissue could reduce in vivo animal trials.
Previous literature reports in vitro models mainly based on non-human cells and hydrogels, which lack structural biomimetic cues, adequate mechanical properties and have fast degradation rate.
The aim of this work was to design mechanically stretchable scaffolds with biomimetic composition for in vitro engineering of human cardiac fibrotic tissue, reproducing different pathological conditions and allowing long-term dynamic testing.
Methodology: Scaffolds were fabricated by additive-manufacturing from polycaprolactone (PCL) providing proper mechanical support and slow degradation rate. Scaffold pores were filled with gelatin methacrylate (GelMA) based hydrogels, mimicking extracellular matrix-like microenvironment1. PCL scaffolds with parallel wavy fibers were fabricated by melt-extrusion additive manufacturing (MEAM).
GelMA hydrogels with different concentrations (5, 7 and 10% w/v) were prepared, using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photoinitiator. Photorheology allowed the definition of the optimal hydrogel curing preserving biocompatibility. Cell viability and protein expression were analysed after encapsulating human cardiac fibroblasts (HCFs) in the different GelMA hydrogels (cell density: 5×106 cells/ml).
PCL scaffolds were functionalized with poly(4-Dihydroxy-DL-phenylalanine) (polyDOPA) to improve interfacial adhesion with GelMA hydrogels. PCL/GelMA scaffolds were then obtained by filling PCL scaffolds with GelMA hydrogels. Static and cyclic tensile tests were then performed on such composite scaffolds. Further in vitro cell characterization is in progress.
Results: Highly stretchable PCL scaffolds were obtained by tailoring PCL fiber diameter and mesh geometry and size, with the aim to mimic cardiac fibrotic tissue stiffness (1-9 MPa) and its maximum elastic deformation (15-22%). PCL/GelMA scaffolds preserved stretchability and integrity after both static and cyclic tensile test. Cell viability tests on GelMA hydrogels showed that UV curing did not damage cells. HCFs were cultured up to 2 weeks in hydrogels and then stained for Phalloidin and α-SMA expression, showing homogeneous cell distribution, spreading and demonstrating HCFs activation into fibrotic phenotype. Collagen Type I and IV content in the cellularized hydrogels was also confirmed after 1 and 2 weeks, demonstrating fibrotic extracellular matrix deposition.

Conclusions: PCL/GelMA scaffolds are promising for cardiac cells culture, such as HCFs, to reproduce post-infarct human cardiac tissue at different severity degrees depending on the stiffness of GelMA hydrogels. Moreover, considering their stretchability, these platforms can be used in dynamic cyclic culture environment (such as bioreactor) to add more complex fibrotic-like stimulus to the model. In the future, such platforms will be also useful for in vitro drug

screening and preclinical validation of cardiac regenerative therapies2, with the advantage to allow long-term dynamic testing.

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keywords: cardiac fibrosis, in vitro modeling, stretchable scaffolds, additive manufacturing, hydrogels.

94238133205

ELECTROCONDUCTIVE PHOTO-CURABLE PEGDA-GELATIN/PEDOT:PSS HYDROGELS FOR PROSPECTIVE CARDIAC TISSUE ENGINEERING APPLICATION

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Introduction
Hydrogels are hydrophilic cross-linked polymeric materials that have been widely studied in tissue engineering (TE) to mimic human tissues [1]. The functionality of electroactive tissues, such as cardiac, neural and muscle, strictly depend on electrochemical signaling between cells. Therefore, TE scaffolds interacting with those tissues should be designed with electroconductive properties [2]. Recently, electroconductive hydrogels (ECHs), combining intrinsically conductive materials with hydrogels networks, have demonstrated to be able to promote the formation of electroactive engineered tissues both in vitro and in vivo.
Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS), a conductive polymer, presents good biocompatibility and dispersibility in aqueous solution. Hence, it has already been involved in the development of ECHs for engineering cardiac or neural tissue [3][4]. Nevertheless, an hydrogel-based scaffold with highly tunable electrical and mechanical properties, showing also bioactivity, biocompatibility and biodegradability, is still missing [2]. The aim of this work, was the development of photo-curable ECHs based on polyethylene glycol diacrylate (PEGDA), gelatin and PEDOT:PSS, with tunable electrical, mechanical and bioactive properties, for cardiac tissue engineering application.

Methodology
In previous studies by the authors, UV-cured hydrogels based on PEGDA and gelatin were obtained [5]. Riboflavin was selected as a biocompatible photoinitiator and three different initial PEGDA/gelatin weight ratios were tested. PEDOT:PSS was incorporated, to impart electrical conductivity to the final system. The photopolymerization process was analyzed through photorheology. Physico-chemical properties of hydrogels were investigated. Mechanical characterization was carried out through compression tests while electrical properties were evaluated by means of sheet resistance and dielectric spectroscopy measurements. In vitro degradation properties of hydrogels were also evaluated. Finally, as a proof of concept for cardiac tissue engineering application, in vitro biocompatibility and adhesion tests with human cardiac fibroblasts (HCFs) were performed on the developed hydrogels.

Results
The gelation time of hydrogels as well as their final cross-linking density, microstructure, swelling and degradation properties were finely modulated by varying the ratio between PEGDA and gelatin. Accordingly, by increasing PEGDA/gelatin ratio, hydrogels with increasing stiffness were obtained, with elastic moduli similar to those reported for healthy native cardiac tissue. The addition of PEDOT:PSS within the hydrogels reduced their gelation time while increasing both their surface and bulk electrical properties. As a fundamental bioactive component, gelatin was successfully bonded to the final hydrogel network structure. Additionally, hydrogels were cytocompatible and promoted the adhesion of HCFs.

Conclusions
Electroconductive photo-curable PEGDA-gelatin/PEDOT:PSS hydrogels developed in this study are promising candidates for cardiac tissue engineering applications, deserving future investigations.

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keywords: hydrogels, conductivity, photo-crosslinking, cardiac, tissue engineering

73296366249

ENGINEERED MODELS OF FIBROTIC CARDIAC TISSUE AS PREDICTIVE PLATFORMS FOR PRECLINICAL VALIDATION

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Introduction
Myocardial infarction (MI) is the main cause of mortality and morbidity worldwide. MI initiates a wound healing process resulting in a fibrotic tissue, characterized by random morphology and increased stiffness [1]. Currently, several advanced strategies based on regenerative medicine are under investigation and in vitro models of pathological cardiac tissue have attracted interest as predictive platforms for their preclinical validation. Previous models of cardiac fibrotic tissue were developed by tissue engineering or organ-on-chip approaches, mainly exploiting cellularized hydrogels[2,3]. However they did not faithfully reproduce the architecture and mechanical properties of cardiac fibrotic tissue. In this context, the aim of this research is to engineer 2D and 3D models of early-stage human fibrotic tissue through bioartificial scaffolds with biomimetic architecture, chemical composition and stiffness.

Materials and methods
Polycaprolactone (PCL) was used as scaffold bulk material; in order to resemble low and high thicknesses of fibrosis 2D random membranes were fabricated by solution electrospinning, while 3D scaffolds with 150 μm and 350 μm square-mesh were built up through melt-extrusion additive manufacturing. Exploiting 3,4-Dihydroxy-DL-phenylalanine polymerization (PolyDOPA), type A gelatin was grafted on PCL to obtain biomimetic surface composition. Scaffold physico-chemical properties were thoroughly investigated. Ventricular human cardiac fibroblasts were cultured on scaffolds up to 3 weeks, to mimic cellular fibrotic microenvironment. Immunofluorescence and two-photon microscopy were used to evaluate the activation of fibrotic cell phenotype and the deposition of pathological cardiac ECM on scaffold area.

Results
Electrospun 2D scaffolds showed homogeneous and defect-free fibers with average diameter of 127 ± 33 nm and pores with lower size than 1 μm. 3D PCL scaffolds (0.7 mm thickness) with 150 and 350 μm square mesh size showed high shape fidelity as suggested by the high similarity between measured and theoretical porosity degree. The progressive formation of gelatin coating mediated by mussel inspired approach was monitored using QCM-D equipment. Immunostaining showed that bioartificial scaffold properties trigger the activation of myofibroblast phenotype and fibrotic-like ECM deposition. Moreover, scanning electron microscopy and two-photon excitation fluorescence show ECM homogeneous distribution on 2D and 3D scaffolds with smaller mesh (150 μm).

Conclusions
In this work, 2D and 3D bioartificial scaffolds, based on PCL and surface functionalized with polyDOPA/G were designed, provided with biomimetic (G coating, isotropic structure and high stiffness) resembling properties of the human cardiac fibrotic tissue, able to support long-term culture of human cardiac fibroblasts, favouring their adhesion, proliferation, differentiation into

myofibroblasts and deposition of ECM on scaffolds. Our results suggest that such innovative in vitro models of human cardiac fibrosis, reproducing patient-specific pathological features, may allow a fine preclinical tuning of new regenerative therapies.

Acknowledgements.
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keywords: In vitro models, bioartificial scaffolds, biofabrication, cardiac fibrosis

62825434808

GENERATION OF A PATIENT-SPECIFIC CARDIAC FIBROSIS MODEL TO ANALYZE LNCRNA CONTRIBUTION TO HEART DISEASE

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Cardiovascular disease is the leading cause of death worldwide, being ischemic heart disease and endomyocardial fibrosis the primary reasons of end-stage heart failure (HF). Cardiac fibrosis is the pathological process mediated by cardiac fibroblasts (cFbs) and determined by the maladaptive remodelling of the heart extracellular matrix (ECM). Although it underlies most of cardiac disfunctions, effective therapies for its inhibition or reversion are currently not available. Long non-coding RNAs (lncRNAs) are potent transcriptional regulators which might be involved in the cardiac fibrotic process and - therefore - have emerged as viable prognosis makers and therapeutical targets. To investigate the role of lncRNAs during fibrotic progression, we differentiated induced pluripotent stem cells (hiPSCs) into cFbs (hiPSC-cFbs) and exposed them to a TGFb-induced fibrotic assay. Following the fibrotic trigger, a subset of fibrosis-related lncRNAs was found dysregulated. A similar trend was observed in primary cultures of cFbs derived from HF patients as compared to the same cells obtained from healthy donors. To better model cardiac fibrosis progression, iPS-cFbs were co-cultured with isogenic hiPSCs-derived cardiomyocytes (iPS-CMs) to generate patient-specific three-dimensional cardiac organoids. This model will be further exploited to test the role of individual lncRNAs by altering their expression through GapmeRs.

keywords: iPS, Cardiovascular disease, lncRNA

PS08
Antimicrobial biomaterials for bone regeneration

83767234686

DROP ON DEMAND PRINTING OF A POLYMER-BASED COMPOSITE RELEASING THE ANTIMICROBIAL PEPTIDE SAAP-148 ON TITANIUM TO PREVENT ORTHOPAEDIC INFECTIONS

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INTRODUCTION: Fracture fixation devices (FFD) have infection rates ranging from 5 to 10% for closed fractures and even higher rates up to 30% for open fractures1 causing a devastating complication. Clinically, implant infections are prevented by systemic antibiotic prophylaxis, the placement of antibiotic laden bone cements, and the use of minimally invasive surgical procedures2. However, conventional biomaterials such as bone cement are compatible with a limited number of antibiotics, and they show poor antibiotic release profiles which can lead to antimicrobial resistant development.
Cationic antimicrobial peptides (AMPs) have shown to successfully kill antimicrobial resistant bacteria. However, the application of AMPs in FFD is quite limited. Therefore, we studied the incorporation of the AMP SAAP-1483 in a polymeric coating printed by drop on demand (DOD) on titanium fixation plates which were 3D printed using the selective laser melting (SLM) technology. These devices were tested in vitro and in vivo in a biomaterial-associated infection mouse model against Staphylococcus aureus.

METHODOLOGY: SAAP-148 was loaded into a polymer-based composite ink and printed by DOD printer on SLM 3D printed titanium coupons and implants. The release of the peptide was carried out in phosphate buffer saline solution at 37°C and 120 rpm. The concentration of the peptide was measured by the µBCA assay. The antimicrobial experiments were performed with S. aureus JAR 06.01.31, an orthopaedic clinical strain. A JIS assay was used to evaluate the surface microbicidal activity of the coating in vitro. The antimicrobial efficacy of the coatings was evaluated in an in vivo subcutaneous biomaterial-associated infection mouse model inoculated with 25 µL containing 106 colony forming units (CFU) of S. aureus, and evaluated by quantitative culture at 1 and 4 days post-surgical infection.

RESULTS: The release assay showed a burst release of SAAP-148 during the first 1 and 24 hours, with 300 µg/mL and 200 µg/mL, respectively, followed by a sustained released of 40 to 60 µg/mL for the next 14 days. Moreover, the coating releasing SAAP-148, exhibited a complete killing in vitro and a significant reduction in numbers of CFU at 1 day post-surgery in the biomaterial and surrounding tissue in the in vivo biomaterial-associated mouse model compared to uncoated titanium.

CONCLUSIONS: The peptide was successfully incorporated in the polymer-based composite showing proper release kinetics and successful antimicrobial activity in vivo. The next step is to evaluate the antibacterial activity of the coating in a bone fixation plate infection mouse model.

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keywords: Antimicrobial peptide, 3D-printing, orthopedic infections

83767259577

FUNCTIONAL E-POLYLYSINE/HYALURONIC ACID HYDROGELS WITH ANTIBACTERIAL ACTIVITY

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Introduction
Large infection spreading rates combined with increasing microbial resistance to antibiotics remain one of the biggest healthcare challenges. In this context, biomaterials based on natural biopolymers with antibacterial properties have recently come to the fore as potential candidate vehicles as both preventative measures and treatments of severe infections. In this study, ε-polylysine (ε-PL), a naturally occurring antibacterial cationic homopolyamide, and a key component of the extracellular matrix – bioactive hyaluronic acid, were both used to synthesize functional composite hydrogels. The aim of this study was to synthesize and evaluate functional and antibacterial activity of the chemically cross-linked hydrogels based on ε-polylysine (ε-PL) and hyaluronic acid (HA) and investigate key interactions from polymer design leading to efficient bactericidal effects.

Methodology
The chemically cross-linked hydrogels were synthesized with ε-PL to HA mass ratios of 40:60, 50:50, 60:40, 70:30 and 80:20 wt%. For covalent linkage between biopolymers 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) cross-linking strategy was used. Physicochemical properties including molecular structure, morphology and mechanical properties of the fabricated ε-PL/HA hydrogels were fully characterized using Fourier transform infrared spectroscopy (FT-IR), gel fraction, scanning electron microscopy (SEM) and oscillatory rheology. Viscoelastic properties were characterized by an amplitude (0.001-1000 % strain interval at 1 Hz frequency) and frequency sweeps (0.01-100 frequency interval at a constant 0.2 % strain). Antibacterial activity was studied against selection of bacterial strains including Gram– (E. coli (ATCC 25922) and P. aeruginosa 9); and Gram+ (S. aureus JAR (010631), S. epidermidis (ATCC 35984) and S. pyogenes (ATCC 19615)). The statistical significance of obtained data was calculated using IBM SPSS software.

Results
FT-IR spectra of prepared ε-PL/HA hydrogels unraveled successful amide bond formation between ε-PL and HA. SEM micrographs revealed formation of three-dimensional networks with highly macro- and microporous homogeneous structure for all synthesized hydrogels. Gel fraction studies indicated that the mean value of the insoluble fraction in the fabricated hydrogels among all ε-PL to HA mass ratios is 55 %. All hydrogels showed larger shear storage moduli (G’) than loss moduli (G’’) in the linear viscoelastic regime (at strains ε=0.01-1%), indicative of a solid-like structure of material. Irrespective of the polymer ratio, on average the chem ε-PL/HA hydrogels exhibited 13.2 ± 3.5 kPa G′ value, indicative of the dominance of the chemical crosslinks over physical entanglements for this type of crosslinking method. During antibacterial studies a positive correlation between increase of ε-PL mass ratio and bacterial inhibition value

was observed. Moreover, starting from ε-PL mass ratio of 50 wt% hydrogel samples showed statistically significant bacteria reduction after 24h contact time ($p<0.05$).

Conclusions
The covalently bonded hydrogels demonstrated highly porous homogenous structure and appropriate viscoelastic properties. Evaluation of antibacterial activity revealed great impact of ε-PL mass ratio on the bacterial inhibition. Antibacterial studies of hydrogel samples revealed both fast (up to 24h) and prolonged (up to 168h) antibacterial effect against Gram-positive and Gram-negative bacteria. Described features open up potentials for the material to be used in tissue engineering (e.g., musculoskeletal disorders or post-surgery site infection treatment).

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keywords: ε-polylysine, hyaluronic acid, hydrogels, antibacterial activity, tissue engineering

94238166507

POLYPHENOLS AND MESOPOROUS BIOACTIVE GLASSES DOPED WITH THERAPEUTIC IONS AS BIOFUNCTIONAL ADDITIVES FOR PCL-BASED COMPOSITES

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Introduction: The growing requirements for tissue engineering results in the design of new materials, including polymer composites with various modifying phases. In addition, active substances can modify the properties of materials, and thus the human body's response. For this purpose, polymer-ceramic composites with various mesoporous glass (MBGs) modifying phases were designed. Numerous literature reports state that ordered mesoporous channels, a large specific surface area and a large pore volume make these glasses ideal candidates as carriers of biologically active substances. In this work, polymer matrix was modified with MBGs doped with therapeutic ions (Cu, Mg). Composites were additionally enriched with sage (Salvia Officinalis L.) extract showing antibacterial, anti-inflammatory and anti-cancer properties.

Methodology: The solvent casting method was used to produce composite films based on PCL. The materials were modified with polyphenolic compounds (PPh) extracted from sage (4.5 wt.%). MBG particles (d50 – 1,5 µm, 30 wt.%) were used as the modifying phase. Composites were analyzed in terms of in vitro bioactivity in simulated body fluid (SBF) by assessment of morphological and chemical changes using SEM/EDX, while kinetics of bioactive layer formation and therapeutic ion release was analyzed using ICP-OES technique. Furthermore, ABTS, DPPH, and FRAP methods were used to evaluate antioxidant activity of composites.

Results and conclusions: All composites showed high bioactivity in contact with SBF. The influence of polyphenols on the formation process of calcium phosphate layer on the surface of materials was noticed. Additionally, ICP-OES analysis showed that the bioactive mesoporous glass made it possible to obtain highly bioactive composite materials capable of releasing therapeutic ions. MBGs affected significantly antioxidant properties of the materials as a result of PPh binding.

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keywords: biocomposites, mesoporous bioactive glass, polyphenols

94238111487

SILVER-DOPED CALCIUM TITANATE LAYER WITH IN VIVO BONE-BONDING ABILITY TO FIGHT BONE BACTERIAL INFECTION IN TITANIUM IMPLANTS

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Introduction
The rapid integration in the bone tissue and the prevention of bacterial infection are key for the success of the implant. In this regard, a silver (Ag) doped thermochemical treatment that generates an Ag-doped calcium titanate layer on titanium (Ti) surface was previously developed by our group in order to improve the bone-bonding ability and provide antibacterial activity 1. However, a greater biological characterization of the proposed material was mandatory. While the incorporation of silver imparts antibacterial properties to the material, this ion can have cytotoxic effects in eukaryotic cells. Thus, the objective of this work is to assess the biological effects of Ag+ in the calcium titanate layer, both in vivo and in vitro.

Methodology
First, cell response in terms of cell adhesion, morphology, proliferation, and differentiation was evaluated using osteoblast-like cells (SaOS-2). Second, the proposed treatment was applied to commercial porous Ti implants (Osteosinter) to study osteointegration in vivo.

Results
The incorporation of Ag is able to inhibit bacterial adhesion without showing any remarkable decrease of viability in osteoblastic cells. Moreover, Ag-doped calcium titanate displays strong bone-bonding ability by promoting extracellular matrix production in vitro. Regarding in vivo results, Ag-doping does not interfere in the outstanding bone-bonding ability of Osteosinter.

Conclusions
According to the results, the Ag-doped calcium titanate layer is an excellent strategy to prevent bone bacterial infection related to implants, which is also able to promote osteointegration.

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keywords: Biomaterial, porous Titanium implants, Silver, antibacterial activity, in vivo

PS09
Biobanking – indispensable support for the development of regenerative medicine

31412754966

DEVELOPMENT OF AN INNOVATIVE DRESSINGS FOR HARD-TO-HEAL WOUNDS: FROM THE BIOBANK TO ADSC-ENRICHED WOUND-CARE PRODUCT

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Introduction:
Cells and tissues are an essential part of many medicinal experiments – either as main players in novel therapies or as a waste material accompanying medicinal intervention (or both). Regardless of their role in therapy they can be collected and stored by biobanks. We present the example of a very effective pathway from a clinical trial of Advanced Therapy Investigational Medicinal Products (ATIMP) containing adipose-derived stem cells towards patent application embracing a cell-containing dressing for treating hard-to-heal wounds. The medicinal area of the clinical trial and the dressing application are completely different (are not related to each other). The point is, that excess cells intended for use in the therapy were collected in the biobank and used in the experiments to design innovative ADSC-enriched wound care products.
With this example, we present a very effective pathway of using the biological material with the biobank in a central part of it.

Methodology
The ADSCs were obtained from healthy donors' subcutaneous fat after informed consent (No KB/887/13, KB/887A/13 and KB/1097/17), then subjected to preparation of ATIMP for the clinical trial under GMP regime. The excess cells were transferred to the biobank of the Medical University of Warsaw and used for development of the innovative dressing. ADSCs were cultured in complete NutriStem medium, then seeded on 4 commercially available wound dressings dedicated for diabetic foot ulcer treatment: Tisseel Lyo, UrgoTul, Mepilex, and MepitelOne. Successful cell attachment to dressings was confirmed with Presto Blue proliferation test, fluorescent staining, scanning electron microscope (SEM), and light microscope (LM) observations. Obtained ADSC-enriched wound dressings were tested for proficiency for wound healing process acceleration in scar tests with fibroblasts from healthy donors (nHF). Additionally, freeze-thaw cycle was performed to confirm the feasibility of long-term storage.

Results:
Both SEM and LM observations confirmed the presence of ADSCs on or in the wound dressings. Presto Blue assay and fluorescent staining showed that ADSC maintain their viability up to 21 days of culture on selected wound dressings. After freezing-thawing cycle ADSC-enriched wound dressings were observed in LM. Only Mepilex did not maintain ADSCs viability throughout the freezing procedure. In the scar test assay, all ADSC-enriched wound dressings accelerated nHF scar closure rate compared to control: UrgoTul–10,30% (starting scar surface), Mepilex–1,32%; Tisseel Lyo–11,78%; MepitelOne–full closure of the scar; no dressing control–20,33%.

Conclusion(s):
In summary, the most promising base for ADSC-enriched ATIMP development were two of the selected commercially available dressings – MepitelOne and UrgoTul. Both setups accelerated wound healing in vitro and maintained ADSC viability throughout the freezing-thawing cycle.

Those are the key features regarding cell-based dressing for hard-to-heal wound treatment. Therefore, the ADSC complexes with MepitelOne and UrgoTul have been subjected to a patent procedure, which resulted in an international patent application – "A dressing for treating hard-to-heal wounds and a process for the manufacture thereof" (PCT application number PCT/PL2021/050068).

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keywords: Stem cells, cell-based therapy, biobank, biobanking, wound dressing

PS10
Biofabricated Tissues and Organs for Clinical Impact

62825422705

A FUNCTIONAL BIODEGRADABLE POLYMER-BASED SEMI- ARTIFICIAL PANCREAS FOR THE TREATMENT OF TYPE:1 DIABETES

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INTRODUCTION: Type 1 diabetes is an autoimmune condition which through pathological immune system action mediates
β-cell destruction, reducing insulin production capacity1. A proposed replacement for insulin injections and pancreas transplants is the production of semi-artificial pancreas, which combines immunoisolative and oxygen permeable polymer hydrogels with mechanically resistant polymer scaffolds. Polyhydroxyalkanoates (PHAs) are becoming known in biomedical applications due to their tuneable properties, non-immunogenicity, and relative biocompatibility with multiple tissue types3. This work aims to generate a 3D printable semi-artificial scaffold for islets, using functionalized bioresorbable bacteria-derived polymers such as alginate and PHAs.

METHODS: The elastomeric medium chain length PHA (mcl-PHA), was produced via bacterial fermentation using a Pseudomonas sp. over a 24-hour period. After centrifugation and freeze drying, the cells were subjected to Soxhlet extraction to obtain pure polymer. The exact chemical structure of the polymer was confirmed using Gas Chromatography-Mass Spectrometry. A multi-material structure comprising BRIN-BD11 beta cell line-encapsulated in alginate, bioprinted into wells in a Fused Deposition Modelling (FDM) printed PHA scaffold. The biocompatibility and functionality of the prototype was measured using the insulin immunoassay, the resazurin viability assay and the LIVE/DEAD assay. VEGF containing microspheres were produced using oil-in-water emulsion method and interspersed throughout the polymer before printing. The revascularisation potential of the resulting constructs was measured using the Chorioallantoic Membrane (CAM) assay.

RESULTS: Initial findings showed that, over 7 days, the cells contained within the mcl-PHA/alginate multi-material scaffolds displayed significantly higher proliferation. compared to cells grown on alginate films, whilst displaying similar glucose-dependent insulin secretion (GSIS). Whilst survival was lower in bioprinted cells on day 1, it was not significantly different to the control by day 7. VEGF/PHA microsphere containing mcl-PHA constructs elucidated a significantly larger vascularisation response compared to the control, displaying significantly greater vascular density.

DISCUSSION & CONCLUSIONS: The cell behaviour analysis demonstrated that BRIN-BD11 cell lines can survive and produce insulin within the mcl-PHA and sodium alginate bioprinted multimaterial scaffolds in vitro, whilst the CAM assay demonstrated the ability of a functionalised construct to induce vascularisation ex ovo. Future work will focus on reducing cell death immediately post bio-printing to counteract the poor proliferative capacity of whole islets. In future, whole islets will be used both in vitro and in vivo, as well as encapsulating a variety beneficial factors within the microspheres for a multimodal benefit to any bioprinted cells.

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keywords: Immunology, Immunoisolation, Tissue Engineering, endocrinology

20941818487

A NEW GENERATION OF TISSUE-ENGINEERED VASCULAR GRAFTS: IMPLANTATION, CONSERVATION AND STERILIZATION.

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To meet the clinical need for small diameter vascular graft, we developed a new generation of human tissue-engineered vascular graft (TEVG) entirely made of biological material. Vessels were produced by weaving yarn of Cell-Assembled extracellular Matrix (CAM). Study objectives were: 1) to assess the graft behavior in the arterial circulation of immunosuppressed rats to evaluate its in vivo functionality, 2) to identify non-denaturing conservation and sterilization methods in order to simplify the manufacturing process.

CAM sheets were produced in vitro by human skin fibroblasts after 8 weeks of culture in DMEM/F-12 with 20% FBS and 0.5 mM Na L-ascorbate and devitalized before use (frozen/airdried). Ribbons (35/TEVGs, 2-mm-wide) were assembled using a custom-made circular loom into 8-mm-long TEVGs with an internal diameter of 1.6 mm. Woven grafts were terminally sterilized with Ethylene Oxide (EtO) and implanted into the abdominal aorta of immunosuppressed rats. TEVGs were explanted after up to 12 months, and echo doppler was performed before sacrifice. Hematoxylin & Eosin and Masson Trichrome (MT) & Verhoeff stanning were performed, as well as smooth muscles and endothelial cells immunostaining. CAM ribbons (5-mm-wide) were conserved in different conditions (-80°C, -20°C, 4°C dry or wet, Room Temperature (RT) dry or wet), and mechanically tested after rehydration after 1 year with a tensile test. CAM ribbons (5-mm-wide) were sterilized using different methods: gamma irradiation at high and low dose rate on dry and wet samples, EtO and supercritical CO2 (scCO2), and compared to control (sterile production). They were subcutaneously implanted in immunodeficient rat for up to 10 months and mechanically tested. HE, MT and Alcian Blue stanning were performed, as well as immunostaining (M1/M2).

Twenty rats were successfully implanted, demonstrating graft implantability and the absence of transmural leakage. After 12 months, US images revealed flow through the graft, and weaving pattern conservation. Preliminary results showed formation of a complete neo-media at 3 months. No or very little cell infiltration in the graft was observed.
After 1 year, only samples conserved dry at 4°C and RT showed a decrease in rehydrated cross section area and an increase in maximum force, reminiscent of the behavior of dry CAM. We believe that those samples had diminished ability to rehydrate, which could be problematic for clinical use. For other groups, no mechanical parameters changed after 1 year. Before implantation, maximum force only decreased for the gamma wet group. In this condition, delamination of ribbons was observed with MT. After implantation, the control group showed a steady max force decrease with time, while the other groups displayed a sharp decrease after 2 months, and then no change. After 10 months, no significant difference in maximum force was observed between groups. Histological analysis is ongoing.

In this study, we showed that our woven TEVG can function for up to a year in the arterial

circulation of rats. We also demonstrated the long-term conservation of our CAM in conditions compatible with hospital storage. Finally, multiple methods remain promising options for graft sterilization.

keywords: tissue-engineering, cardiovascular, blood vessels

94238167059

BIOINK WITH CARTILAGE-DERIVED EXTRACELLULAR MATRIX MICROFIBERS ENABLES SPATIAL CONTROL OF VASCULAR CAPILLARY FORMATION IN BIOPRINTED CONSTRUCTS

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Microvasculature is essential for the exchange of gas and nutrient for most tissues in our body. Some tissue structures such as the meniscus presents spatially confined blood vessels adjacent to non-vascularized regions. In biofabrication, mimicking the spatial distribution of such vascular components is paramount, as capillary ingrowth into non-vascularized tissues can lead to tissue matrix alterations and subsequent pathology. Multi-material three-dimensional (3D) bioprinting strategies have the potential to resolve anisotropic tissue features, although building complex constructs comprising stable vascularized and non-vascularized regions remains a major challenge to date. In this study, we developed endothelial cell-laden pro- and anti-angiogenic bioinks, supplemented with bioactive matrix-derived microfibers (MFs) that were created from type I collagen sponges (col-1) and cartilage decellularized extracellular matrix (CdECM), respectively. HUVEC-driven capillary networks started to form two days after bioprinting. Supplementing cartilage-derived MFs to endothelial-cell laden bioinks reduced the total length of neo-microvessels by 29%, and the number of microvessel junctions by 37% after 14 days, compared to bioinks with pro-angiogenic col-1 MFs. As a proof of concept, the bioinks were bioprinted into an anatomical meniscus shape with a biomimetic vascularized outer and non-vascularized inner region, using a gellan gum microgel suspension bath. These 3D meniscus-like constructs were cultured up to 14 days, with in the outer zone the HUVEC-, mural cell-, and col-1 MF-laden pro-angiogenic bioink, and in the inner zone a meniscus progenitor cell- and CdECM MF-laden anti-angiogenic bioink, revealing successful spatial confinement of the nascent vascular network only in the outer zone. Further, to co-facilitate both microvessel formation and meniscus progenitor cell-derived matrix formation, we formulated cell culture medium conditions with a temporal switch.
Overall, this study provides a new strategy that could be applied to develop zonal biomimetic meniscal constructs. Moreover, the use of ECM-derived MFs to promote or inhibit capillary networks opens new possibilities for the biofabrication of tissues with anisotropic microvascular distribution. These have potential for many applications including in vitro models of vascular-to-avascular tissue interfaces, cancer progression, and for testing anti-angiogenic therapies.

keywords: cartilage matrix microfibers, extrusion-based bioprinting, meniscus construct

20941822555

DESIGN OF AN ADVANCED THERAPIES CLINICAL TRIAL FOR THE EVALUATION OF A NOVEL SUBSTITUTE OF THE PALATE MUCOSA IN CLEFT PALATE CHILDREN

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Introduction: Cleft palate is a congenital condition affecting the development of maxillofacial bones. Current treatment is based on the surgical repair of the soft tissues in order to generate a physical barrier between the oral and nasal cavities. However, this treatment is associated with subsequent bone hypoplasia and misdevelopment in most patients. In this regard, we recently generated a bioengineered model of the human palate mucosa using fibrin-agarose biomaterials that offered promising results in newborn rabbits1,2. These positive results allowed us to design an advanced therapies clinical trial (BIOCLEFT) in which autologous palate mucosa will be grafted in a group of patients at the moment of the surgical repair of the palate defect.

Methodology: First, small oral mucosa biopsies obtained at the moment of the cleft lip repair, are processed to generate primary cell cultures of keratinocytes and fibroblasts. Then, bioengineered substitutes of the human palate mucosa are generated using fibrin hydrogels with 0.1% agarose.

Results: Application of the biofabrication methods optimized by the research group allow the efficient generation of palate mucosa substitutes in the laboratory. Palate mucosa substitutes generated by tissue engineering are viable and show structural similarities with native human palate, with a stroma substitute containing fibroblasts and a stratified epithelium on top. This palate mucosa substitute will be used to cover the maxillary bone defect of patients corresponding to the BIOCLEFT study group, whereas the standard surgical technique in which the bone defect is left uncovered is used in control patients. Results will be first analyzed in

terms of feasibility and safety and then, in terms of improvement in craniofacial growth and development.

Conclusions: These bioengineered palate mucosa substitutes fulfil the criteria for use as ATMP for the regenerative treatment of children with cleft palate. The positive results obtained in laboratory animals in terms of biosafety and functionality support the putative beneficial effect of this technology in the BIOCLEFT clinical trial.

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keywords: Cleft Palate, Regeneration, Traslatión

31412754248

USING OF PERFUSION BIOREACTOR FOR DYNAMIC CULTURE OF ADIPOSE DERIVED STROMAL CELLS ON TUBULAR SCAFFOLDS – AN IN VITRO STUDY

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Introduction:
Bladder cancer is the 10th most common cancer worldwide. About 20% of bladder cancers are muscle-invasive and are indication for a radical cystectomy. The use of ileal segment is a standard method for urinary diversion after bladder removal. Use of this method extend surgical procedure and lead to numerous metabolic complications. To overcome these side effects, tissue engineering methods can be utilized to create an artificial urinary conduit. The aim of this study was to develop a method of the tissue-engineered conduit construction for urinary diversion using dynamic bioreactor culture.

Methodology:
Tissue engineered tubular scaffolds were used for construction of the artificial urinary conduits (n=40). Conduits were seeded manually with adipose-derived mesenchymal stromal cells (~10x106/cm2) isolated from porcine fat tissue. Cells were grown on scaffold surface in a bioreactor for 6 days. During the culture pH, pO2 and temperature were continuously monitored. On the basis of culture parameters and glucose values measured each day medium feeding rate was established. ADSCs were seeded after 3rd or 4th passage, before seeding on scaffold mesenchymal stromal cell phenotype was analyzed using flow cytometry (positive markers: CD29, CD44, CD90; negative markers: CD11b, CD31, CD45). After end of experiment analysis of cell growth on scaffold surface was performed using scanning electron and confocal microscopy. Cells were detached from scaffold surface in order to check their number and changes in phenotype after dynamic culture in bioreactor.

Results:
ADSCs expressed typical for mesenchymal stem cells markers. Scanning electron microscopy revealed appropriate morphology of cells grew on artificial urinary conduit. Cells were elongated, spindle-shaped with good condition confirmed by production of microvesicles. Analysis using confocal microscopy showed that majority of cells seeded on artificial urinary conduit after the end of 6-days culture were viable. Analysis of culture medium and bioreactor

vessel showed that only small number of cells (<10%) detached from tested material during dynamic culture in bioreactor. Cells detached from scaffold surface showed significant changes in cell phenotype in the case of CD29 antigen.

Conclusion:
Dynamic culture of tissue-engineered scaffold seeded with ADSCs in bioreactor enables their proper growth. Application of such culture method may allow for construction of artificial organs for clinical application.

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keywords: AD-MSC, bladder cancer, urinary conduit, tubular scaffold

20941861677

VASCULARIZED 3D IN VITRO SKIN MODEL

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For almost a century, in vitro 3D tissue models of skin are known. However, these 3D skin models are mainly used for basic cosmetic testing rather than for clinical application. This is because of a lack of reproducible methodology, physiological structures and tissue architecture. An essential step in the development of a physiologically relevant in-vitro skin model is the incorporation of functional blood vessels (BVs) and high longevity. The advent of current tissue engineering strategies has now facilitated the fabrication of vascularized human skin equivalents (vHSEs). However, the challenge of maintaining the stability and durability of the developed vHSEs for a long duration persists. Unfortunately, the cause and the solution for the instability of both vascularized and non-vascularized HSEs are seldom reported in the literature. An important aspect that is overlooked during the construction of vHSE is the role of keratinocytes (KCs), the cells forming the epidermis. It is known that KCs modulate the balance between the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) as well as secrete angiogenic growth factors such as vascular endothelial growth factor (VEGF). BVs in turn respond to such factors and undergo rapid branching or regression depending on the signal. Therefore, the cross-talk between KCs and the vascularized dermis in-vitro results in a vicious cycle of vascular regression and matrix degradation. In the context of the skin model, this has never been investigated. Can a flow culture ameliorate this?
Previous reports have mostly elucidated the role of dynamic cultures on the improvement of the barrier properties of skin model or in a few cases vasculature but they fail to show in entirety why a dynamic flow environment can sustain a long-term vessel architecture, dermal integrity and thereby maintaining vHSEs homeostasis.
In this work, we report the fabrication of scaffold-free vHSEs cultivated within a 3-D printed flow bioreactor to mitigate the uncontrolled formation of BVs in the vHSEs. This was achieved by the modulation of VEGF and hypoxia inducible factor 1A (HIF1A) gene expression and by maintaining the balance of MMPs/TIMPs gene expression, thereby, improving the vHSEs stability. Apart from the enhancement of barrier properties, optimal epidermal differentiation and improved dermal stability, flow culture also resulted in the formation of perfusable vascular openings, which could be attributed to the oscillatory flow patterns and growth factor gradient. As an example of the application of flow culture, we conducted a 3D wound healing assay to show the effect of flow in comparison to the static culture. Together, this work deliberates the response of lab-grown vHSEs to a perfusable flow environment and emphasizes the requisite of dynamic tissue-engineered strategies for improved vascularized skin constructs.

keywords: skin, vascularization, in vitro, organ model

PS11
Biofabrication using extrinsic fields

52354558037

MAGNETICALLY-GUIDED CARTILAGINOUS MICROTISSUES ENABLE BIOFABRICATION OF IMPLANTS USING IRON OXIDE MAGNETIC NANOPARTICLES

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Introduction
The rise of spheroid-based tissue engineering has resulted in efficient strategies for skeletal defect regeneration through the formation of cartilage intermediate templates (1). As previously demonstrated, spheroid-based implants composed of cartilaginous microtissues can form ossicles upon implantation and regenerate critical size tibial defects (2). However, these implants were formed through microtissue self-assembly, and the formation of dense spheroid-based implants is a challenge through conventional extrusion based bioprinting. Novel, nozzle-free rapid biofabrication method for engineering large microtissue-based implants need to be developed. In this work, we explore the use of two types of iron oxide nanoparticles (IONPs), their loading on cartilaginous microtissues and their potential as magnetically active building blocks for the biofabrication of callus-like mesotissues.

Methods
Human periosteum-derived cells (hPDCs), after 7 days of 2D expansion in DMEM complete medium were seeded in non-adherent microwells (AggreWell™400, STEMCELL Technologies) to form 3D spheroids. The spheroids were then cultured with either a chemically defined differentiation chondrogenic medium (CM) or basal medium for 7 days and then were incubated overnight with 0.5 μgr/mL dextran or citric acid coated IONPs. The treated spheroids were eventually placed in non-adherent 24 well plates and upon applying magnetic field, they were guided into forming a meso tissue (d=2 mm) which exhibited growth in size during culture (in chondrogenic medium) after 8 days. Microscope images were obtained at different timepoints, and non-treated spheroids were used as a control. Finally, image analysis was used for the quantification of implant's growth.

Results
Following the characterization of the IONPs by TEM, XRD and FT-IR, spheroids cultured in CM had approximately 200 μm in diameter where the spheroids cultured in basal medium had an approximate diameter of 100 μm. Dextrose and citric acid coated IONPs after been incubated with the spheroids, they offered their magnetic actuation properties to the cartilaginous microtissues and thus they were able to respond to an external magnetic stimulus and be guided. The spheroids cultured with CM formed a granular mesotissue after the magnetic biofabrication, while spheroids cultured in BM formed a mesospheric tissue. In both samples, CM was used after the bioassembly and in both cases they doubled their size after 8 days in culture indicating that cells survive the process of biofabrication.

Conclusions
This biofabrication approach paves the way for a rapid and low shear method to produce scaffold-free implants formed from magnetically loaded cartilaginous microtissues under the guidance of a magnetic field. This approach overcomes limitations such as loss of microtissues

during their handling in cell culture and moreover minimizes the time required for the biofabrication process in contrast to extrusion-based bioprinting.

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keywords: spheroids, magnetic guided biofabrication, iron oxide nanoparticles (IONPs)

PS12
Biofabrication with light-based technologies and high-definition printing

20941806129

3D PRINTABLE SELF-HEALING HYDROGEL AND INJECTABLE CRYOGEL BASED ON GELATIN AND POLYURETHANE

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3D-Printable biomaterials have potential for fabrication of customized scaffolds for tissue engineering. In the study, methacrylated gelatin (GelMA) and aldehyde-functionalized polyurethane (PU) were synthesized and used to prepare 3D-printable GelMA-PU biomaterials. The GelMA-PU hydrogel and cryogel were optimized for compositions and manufacture processes. The GelMA-PU hydrogel was 3D-printable through a 210 μm nozzle. The separately printed modules could be further assembled into constructs of complex structure owing to the self-healing ability, and the integrated structure was stabilized by secondary photo-crosslinking. The self-healing ability of the hydrogel was switched off after photo-crosslinking while the resolution was maintained. The GelMA-PU hydrogel embedding human bone marrow mesenchymal stem cells (hMSCs) was successfully printed and grown for 14 days. Besides, 16-gauge needle injectable cryogel with high compressibility was acquired by adjusting the composition of GelMA/DFPU and freezing. The latter cryogel was 3D-printable through a programmed low-temperature printing procedure. The 3D-printed GelMA-PU cryogel scaffolds displayed high swelling ratio and remained the high compressibility. hMSCs seeded in the 3D-printed GelMA-PU cryogel scaffolds also displayed long-term growth in 14 days. The GelMA-PU composite systems demonstrate the versatility as both hydrogel and cryogel, which have potential applications in 3D bioprinting, tissue engineering, and minimally invasive surgery.

keywords: 3D printing, self-healing hydrogel, cryogel, GelMA, polyurethane

20941818004

DIGITAL LIGHT PROCESSING OF POLYESTER-BASED MATERIALS AS AN ALTERNATIVE ROUTE TOWARDS PATIENT-SPECIFIC BREAST RECONSTRUCTION

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According to estimations of the Global Cancer Observatory, over 2.2 million women were diagnosed with breast cancer in 2020, worldwide. [1] While most often requiring surgical removal of the affected breast tissue, breast reconstruction following mastectomy remains a challenge. [2] Conventional reconstruction techniques, using synthetic prostheses or autologous tissue, are accompanied with risks and complications such as infection, prosthesis rupture, capsular contracture, donor site morbidity, uncontrollable resorption rates and the need for reinterventions. [3,4,5] Therefore, a strong research focus is directed towards regenerative adipose tissue engineering, shifting reconstruction strategies from tissue replacement to autologous tissue regeneration.

In the present work, a novel approach is introduced to enable minimally invasive, patient-specific, aesthetically elegant breast reconstruction. Shape memory copolymers based on aliphatic polyesters were synthesized and functionalized into diacrylate and hexaacrylate end-capped urethane-based polymers (AUPs) to enable chemical crosslinking. The materials were tuned in order to achieve a glass transition temperature below body temperature. The AUPs were characterized physico-chemically by proton nuclear magnetic resonance (1H-NMR) spectroscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

Subsequently, a photo-sensitive resin was developed based on the synthesized AUPs, rendering them suitable for digital light processing (DLP). By varying the molar mass of the polymer, the acrylate content of the AUPs and the concentration of photoinitiator and photoabsorber, the resin was optimized towards efficient photo-crosslinking. Furthermore, the gel fraction and swelling ratio were determined. Once optimized, the photo-sensitive resin was processed into porous scaffolds by means of DLP. The morphology of the scaffolds was investigated using optical microscopy and scanning electron microscopy. Suitable pore sizes were obtained for diacrylate AUPs, whereas for hexaacrylate AUPs further optimization is required.

Preliminary in vitro biocompatibility assays using adipose-derived stem cells were performed to assure the absence of cytotoxic components. The cytotoxicity was tested through an indirect assay in which the crosslinked materials were submerged during 24h, 72h and 7 days in culture media. The culture media, containing any leached components, was placed on top of the seeded cells in order to assess biocompatibility through a live/dead staining and MTS assay.

Finally, future experiments include (accelerated) degradation assays as well as mechanical characterization of the 3D printed scaffolds. Additionally, the shape memory properties will be investigated, as these will enable a shift in shape from a temporary reduced size into a permanent large, patient-specific volume upon implantation in the body, assuring minimally

invasive surgery.

As a result, the developed porous shape memory scaffolds can be considered promising candidates towards minimally invasive, patient-specific adipose tissue engineering.

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keywords: Shape memory polymers, Adipose Tissue Engineering, Digital Light Processing

31412758408

GELMA/NHA BIOMATERIALS INK FOR BONE TISSUE IN VITRO MODELS

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Introduction
Bone tissue is a vascularized, highly specialized, and hierarchically organized tissue. Nowadays, Tissue Engineering is challenging to recapitulate the structural complexity of physiological bone extracellular matrix (ECM), and biomaterials have been widely investigated [1]. Herein, methacrylated gelatin (GelMA) was largely involved since physico-chemical and mechanical properties of GelMA-based hydrogels can be finely tuned. For this purpose, a biomaterial ink for bone 3d in vitro models was here designed and developed, by adding hydroxyapatite nanoparticles (nHA) in GelMA solution, photocuring with visible light exposure [2].

Materials and Methods
GelMA was properly methacrylated and then dissolved (7.5% w/V) in DPBS with Ruthenium/Sodium Persulfate as photoinitiator. nHA, added to mimic bone inorganic composition, was added in different concentrations. Composite solutions were tested by shear rate ramps (0.01-1000 s-1) to assess biomaterial ink viscosity with a rheometer. Hydrogels were consequently obtained by exposing the solution to light, mechanical compressive properties were tested, and weight variations in distilled water were evaluated for up to 6 weeks. The optimized biomaterial ink was investigated in terms of printability with a pneumatic extrusion-based BIO X 3D printer, to optimize printing parameters (i.e., speed, pressure, nozzle gauge). Serpentine models and grids were printed to assess printing accuracy (PA%) [3]. In vitro biological tests were performed by encapsulating SAOS-2 preosteoblastic cells in the nHA/GelMA, and cell viability (%) was qualitatively evaluated by LIVE/DEAD staining.

Results
GelMA methacrylation was successfully checked by H NMR spectroscopy (DoF = 55.32%). Rheological analysis showed that the investigated formulations exhibited a typical shear-thinning behavior, adequate for biomaterial ink printability. Viscosity increased by enhancing nHA concentration since nanoparticles acted as a filler. Contrarily, Ru/SPS addition led to a viscosity decrease in solutions with higher nHA concentration, possibly due to unfavorable electrostatic interactions between Ru/SPS and nHA. Stability tests exhibited a weight decrease in the first hours of water immersion, due to the release of uncrosslinked polymeric chains. By adding nHA, higher Ru/SPS concentration led to a higher swelling since a denser polymeric network allowed for the nHA exclusion, and samples absorbed higher water quantity. Contrarily, lower Ru/SPS concentration formed hydrogels whose meshes retained nHA and determined a lower water uptake. Mechanical tests showed higher Ru/SPS concentration led to a decrease ($p < 0.05$) in mechanical properties compare to GelMA, validating the weight variations results. Biomaterial ink printability was assessed by printing serpentine, to select optimal parameters set, and grid models as well, showing a PA% equal to 65%. High viability (> 80%) was qualitatively checked by

LIVE/DEAD staining up to 7 days of culture when SAOS-2 cells were embedded in the hydrogel.

Conclusions
The developed biomaterial ink resulted in a promising biomaterial to better recapitulate bone tissue ECM. Printability of the materials here developed was successfully evaluated by rheological analysis and printability tests. Moreover, cells showed good viability up to 7 days of culture.

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keywords: Gelatin, ink biomaterial, bioink, bone model, nano hydroxyapatite

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PHOTOINITIATOR- AND RADICAL-FREE HIGH RESOLUTION BIOPRINTING

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Introduction. In recent years, the development of novel photo-crosslinking strategies and photoactivatable materials has stimulated a widespread use of light-mediated biofabrication techniques.1 From the high resolution two-photon stereolithography (2P-SL),2 to the novel fast volumetric printing,3 light-based technologies now encompass a powerful set of tools that enables one to mimic multiscale tissue organization with sub-micrometer resolution and centimeter-size scale.
However, despite great improvements towards more efficient and biocompatible photochemical strategies, current photoresins still rely on the presence of photoinitiators (PIs). Upon light exposure, PIs produce radical-initiating species to trigger the crosslinking/polymerization process. In the context of bioprinting where cells are encapsulated in the photoresin, the presence of radicals raises concerns of potential cytotoxicity. On the other hand, near-UV and visible light alone were shown to be biocompatible,4 thus suggesting that the use of a photoinitiator/radical-free photochemistry would be an ideal approach. In this work, we present a universal radical-free crosslinking strategy to be used for light-based technologies.

Methodology. A water-soluble coumarin photocage (DCMAC-OH) was synthesized as previously reported,5 and exploited to mask thiol groups of a thiol-terminated PEG crosslinkers (PEG2SH, PEG4SH). Stability in solutions and dry condition of such crosslinkers were determined by 1H-NMR. Synthetic and natural-derived polymers were modified to bear -ene functionalities (vinyl sulfone, methylsulfone, maleimide). The thiol and -ene component of the photoresin were combined in 1:1 thiol:ene molar ratio and photocrosslinking was monitored with photorheology under oscillation. Potential toxicity of DCMAC-OH was investigated with the MTT assay using primary cells and immortalized cell lines. Photoresins were adopted for high-resolution two-photon stereolithography performed on a LeicaSP8 equipped with MaiTai fs-laser.

Results and conclusions. The radical-free crosslinking method presented in this work relies on base-catalysed thiol-ene chemistry. Due to the caging of thiol groups with the one- and two-photon sensitive photocage DCMAC-OH, crosslinking can be triggered with multiple light-based technologies using 365 nm, 405 nm or two-photon lasers. As demonstrated by photorheology, upon light absorption the photocage is removed and the thiols of the PEG-based crosslinker are free to react with any polymer functionalized with -ene moieties, thus making our method universally applicable. In this work we have adopted three different -ene groups, the widely used maleimides and vinyl sulfone, and a recently discovered phenyl-tetrazole-based methylsulfone.6 Photorheology analysis has shown that reaction with vinyl sulfones is too slow for light-mediated bioprinting, while maleimides and methylsulfones reaction kinetics could be adopted for such methods. The PI-free photoresins were successfully employed for high-resolution 2P-SL. Viability and proliferation assays demonstrated the cytocompatibility of the photocage for stem cells, primary cells and immortalized cell lines. Stability tests of the photocaged crosslinker confirmed excellent stability for up to 6 months, making it a potentially suitable platform for commercialization and off-the-shelf preparations. Our universal PI/radical-free method could represent a paradigm-shift in the light-based technologies, avoiding the need to expose encapsulated cells to harmful radicals.

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keywords: radicals, photoinitiator, bioprinting, thiol-ene, two-photon

94238149955

UV-CURABLE POLYMER AND NANO HYDROXYAPATITE INKS FOR MULTI-MATERIAL 3D INKJET PRINTING FOR TISSUE ENGINEERING

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Introduction
3D inkjet printing provides a vital tool to deposit biomaterials with high precision (in the micrometer range) to address the complexity required for 3D scaffolds fabrication. However, its implementation is hindered by the lack of inks that exhibit properties optimal for tissue regeneration, and meet the properties for the demanding inkjet printing process. We address this challenge by engineering a hydroxyapatite nanoparticle ink (nHAp) as well as a UV-curable biodegradable polymer (BDP) based on thiol-yne click chemistry.
nanoXIM (Fluidinova), is a synthetic aqueous hydroxyapatite suspension containing rod-shaped nanoparticles. It has been used to produce bone regenerative materials and is biocompatible and osteoinductive. However, its suitability as an inkjet ink has not been proved yet.
The BDP system comprises of 4 monomers; a trifunctional thiol crosslinker, an aliphatic di-alkyne ether, a viscosity modifier and a phosphorus-based monomer. This phosphorus monomer is based on a highly versatile system and we developed a monomer library which enables us to create BDPs with a range of properties (mechanical, degradation behaviour).

Methodology
Several formulations of nHAp and polymer inks were developed and their viscosity and surface tension were systematically evaluated using a viscosimeter or rheometer and a Drop Shape Analyzer. Those formulation meeting the properties for the inkjet process were then tested in a lab printer (DIMATIX DMP2800). In case of the polymer ink, the formulation was also tested with an industrial printhead (Pixodro LP50 with a Spectra SL128).
The stability of nHAp formulation was evaluated by means of long-term sedimentation behavior using Turbiscan equipment. The solid content was determined gravimetrically. The degradability of the monomers and cured BDP is analyzed by NMR spectroscopy and mass loss tests.

Results
nanoXIM resulted in a promising material for inkjet inks after tuning its viscosity, surface tension and agglomeration behaviour with additives. nHAp ink was stable for at least three weeks. Some phase separation was observed which could be avoided by using recirculating ink supply systems. nHAp solid contents of the inks varied from 2 to 8 %wt. The ink can be used as a coating, therefore different patterns were successfully inkjet printed on top of the BDP.

3D features including pillars, holes and walls were successfully printed with the UV-curable polymer formulation without phosphorus monomers using an industrial printhead. Degradation tests confirmed that the addition of phosphorus monomers/mixed formulations lead to a degradable polymer formulation.

Conclusions
Two new experimental inks were successfully developed and their suitability for an inkjet

process was demonstrated. Already proven biocompatible nHAp suspensions were formulated into an inkjet ink and used on a lab scale printing process. The potential for upscaling was demonstrated opening the way to the use in an inkjet industrial process. Several new BDP ink formulations were tested. One of them shows very good performance in a 3D inkjet printing process using industrial printheads. The degradation behaviour can be tuned with the addition of phosphorous monomers. Compatibility of the two inks is demonstrated. Further development of the use in a 3D multi-material process is ongoing.

I want my abstract to be considered for a poster presentation.

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keywords: 3D inkjet printing, nano-hydroxyapatite, biodegradable polymer, ink

PS13
Biofunctionalized surfaces for cellular and tissue engineering

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A COMPUTATIONAL MODEL FOR THE RELEASE OF BIOACTIVE MOLECULES FROM A FUNCTIONALIZED SCAFFOLD

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Introduction
This work presents a computational model to study release profile of proteins grafted on scaffolds surfaces. As a case study we investigated the behaviour of ICOS-Fc, a bioactive protein able to rebalance the osteoblasts and osteoclasts activities [1], bound to a polyester blend. Then, a three-dimensional (3D) model was implemented to evaluate the release and diffusion of ICOS-Fc, from the pores of a complex scaffold towards an osteoporotic fracture.

Methodology
The protein release profile was simulated in COMSOL Multiphysics® software implementing the "Transport of Diluted Species" equations. A first simplified model was implemented to study the protein release from the surface of cylindrical pellet incubated in a PBS medium, in order to identify the process parameters. The protein was assumed completely grafted and homogeneously distributed on the pellet surface. Thus, considering the axial-symmetry of the geometry and the problem conditions, a 2D axisymmetric model and a time dependent study were selected to assess the protein release over time. A parametric sweep was added to evaluate the release profile for different diffusion constants(10-9 - 10-8 - 10-7 m2/s [2]).An initial concentration of 0.2 mol/m3 was set on the layer where the protein is grafted, while 0 mol/m3 on the medium domain. On the basis of the parameters obtained from the simplified study, a 3D problem was modelled for the protein release grafted from a 3D-printed complex scaffold, and its diffusion from the pores towards a fracture. An initial concentration of 0.2 mol/m3 was set on the scaffold internal surface where the protein is grafted. A flux condition was set on the delivering pores to simulate the diffusion of the molecule. In addition, the protein release profile was studied changing the scaffold porous architecture. A time dependent study was chosen to evaluate the release after 7 days.

Results
The amount of protein released from the scaffold, with all and four pores delivering pores over time, was computed. Comparing the release profile of the two cases, in the first one the protein is almost all released after 4 days. Instead, with only four pores delivering, the protein is not all delivered after 7 days. Thus, the second condition is preferred for a controllable release of the protein.

Conclusion
To sum up, the presented study showed a computational model for evaluating the ICOS-Fc protein release over time. With the process parameters studied in the simplified study, the model can be used to optimize the scaffold porous architecture in order to have a controllable release of the protein (e.g., all or only four pores delivering).

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keywords: scaffold, biofunctionalization, modelling

73296349239

BIOMIMICKING POLYISOCYANIDE-HYDROGEL TO IMPROVE VAGINAL FIBROBLAST FUNCTION IN PELVIC FLOOR REPAIR

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Introduction: Pelvic organ prolapse (POP) is a condition in which defects of supportive tissues due to changes in collagen metabolism result in bladder, bowel, and uterus descending into the vagina, which results in a lifetime surgery risk of 10% worldwide [1]. Surgery comprises the plication of healthy connective tissue to cover the defect, which is called native tissue repair (NTR). However, the extracellular matrix (ECM) deposition and remodeling by fibroblasts in the tissue are already comprimised leading to a high failure rate. We hypothesize that the synthetic but strongly biomimetic thermosensitive polyisocyanopeptide (PIC)-based hydrogel [2] could be applied to the surgical site to deliver wound-healing promoting factors. To that end, PIC was decorated with cell-adhesive ligands containing the Arg-Gly-Asp (RGD) that allow for cell-matrix interactions. Here, we studied wound healing effects in terms of collagen, elastin and collagen-cleaving enzymes (MMP-2) for vaginal fibroblasts seeded on PIC gels, with different physical properties and RGD densities.
Methodology: In vitro experiments (n = 3) were performed using vaginal fibroblasts from POP tissue cultured on PIC hydrogels with RGD in 50 µM (PIC-RGD50) and 180 µM (PIC-RGD180) and without RGD (PIC-RGD0)[3]. Cell behavior and functionality were assessed from their morphology (fluorescent imaging of nucleus (Hoechst) and actin filaments (Phalloidin)), proliferation (WST-I assay) and ECM metabolism on the protein level [elastin (Fastin assay), collagen (picrosirius red), and MMP-2 production (zymography)] and gene level (quantitative PCR) up to 28 days of culture. As an illustration, data at day 21 is presented.
Results: After 4 days of culture, cell morphology images showed interconnected cell networks on PIC-RGD180, cell aggregates with elongations on PIC-RGD50, and round clusters appeared on PIC-RGD0. Vaginal fibroblast proliferation increased 2.5-fold on PIC-RGD180 ($p < 0.01$) and PIC-RGD50 ($p < 0.005$) compared to PIC-RGD0 at day 21. On PIC-RGD180, collagen (+60%, $p < 0.005$) and elastin (+40%, $p < 0.01$) deposition were significantly increased compared to PIC-RGD1% as well as active MMP-2 albeit not significantly (+20%, $p > 0.05$). Gene expression for collagen I, III, elastin and MMP-2 were in line with the protein results. The cells cultured on PIC-RGD0 did not produce these ECM factors at any detectable level.
Conclusion: The presented results show that PIC-hydrogel with RGD-ligand triggers regenerative behavior by vaginal fibroblasts in terms of collagen, elastin, and MMP-2 production, which are crucial factors in the healing process of pelvic connective tissue after POP surgery. We aim to apply PIC-RGD180 in vivo to study the host response and performance of PIC-hydrogel in NTR.
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keywords: Pelvic organ prolapse, Vaginal fibroblasts, Hydrogel, RGD, Extracellular matrix

73296321006

DENTAL ABUTMENT SURFACES BIOFUNCTIONALIZED BY HYDROLYTICALLY STABLE CROSS-LINKED PROTEINS PROMOTE ENHANCED ADHESION, PROLIFERATION, AND MIGRATION OF GINGIVAL CELLS

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Introduction
In addition to the osseointegration of the implant's surface, a sufficient adhesion of the abutment's surface to the soft tissue is a crucial factor for the longevity of the implant. The soft tissue seal surrounding natural teeth acts as a barrier against deleterious stimuli in the oral cavity, thus preventing bacteria penetration and protecting the underlying alveolar bone. However, the peri-implant mucosa around the abutment is different from the mucosa around natural teeth, which can result in a poorly bacterial resistance. To overcome this limitation, this study proposes a novel method to biofunctionalize abutment material surfaces through the covalent conjugation of specific proteins to enable a stable soft tissue adhesion.

Materials and Methods
A silane monolayer was applied on the established abutment materials (Y-TZP and Ti6Al4V) with subsequent coupling of proteins of the extracellular matrix (ECM), fibronectin and laminin, via a bifunctional crosslinker. The successful application was proved by XPS, AFM, and immunostaining. Preserved biofunctionality of the ECM proteins after surface coupling was shown in a centrifugation-assay. Proliferation, cell adhesion, and migration behavior of human gingival cells on the ECM-modified and non-modified specimens as a control was investigated additionally. Moreover, integrin expression of gingival cells on the individually modified surfaces was determined via confocal microscopy and flow cytometry. Stability of the applied substances on both specimens was tested via mechanical exposure, acid and heat resistance and was examined using XPS, SEM, and AFM.

Results
The centrifugation-assay showed that gingival cells seeded onto the ECM-protein-coupled surfaces exhibited significantly higher adhesion ($p < 0.001$) in comparison to non-functionalized controls. In addition, a proliferation assay (CCK-8) showed that significantly more cells ($p < 0.05$) were evident after seven days on ECM-modified surfaces compared to non-functionalized surfaces. Confocal microscopy revealed a much higher cell area (up to threefold) and enhanced expression of pFAK-Y397 and vinculin on ECM protein-coated surfaces compared to the controls. Moreover, the wound healing assay demonstrated, that cells seeded on ECM-modified surfaces exhibited significant more migration activity compared to untreated specimens. Stable attachment of the ECM proteins over 21 days was confirmed via ELISA.

Conclusion
Covalent conjugation of ECM proteins by cross-linking on the abutment material surfaces Y-TZP and Ti6Al4V enables improved adhesion, proliferation, and migration of human gingival cells. Therefore, in future clinical applications, this novel approach could lead to improved soft tissue adhesion and thus potentially prevent bacterial penetration through the soft tissue-abutment interface. Finally, this biofunctionalization approach could contribute to reduce or even prevent peri-implant diseases.

Acknowledgments
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keywords: dental abutment, functionalization, cell adhesion, peri-implant disease

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ELECTROACTIVE SA/PCL HYDROGELS WITH CONDUCTIVE RGO NANOPARTICLES FOR MUSCLE TISSUE ENGINEERING

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Abstract
Tissue engineering (TE) combines both cells' biology and engineering to develop bioactive biomaterials to treat tissue regeneration [1]. Among them, biomaterials with electric conductive properties have shown promising potential as bioactive cell substrates for tissue regeneration, especially for electrically active tissues, such as the skeletal muscle system [2].
Alginate hydrogels possess excellent properties and biocompatibility for a broad range of biomedical applications [3,4]. Nevertheless, they present weak mechanical properties within a few hours in physiological solution [5]. In addition, the hydrophilic nature of alginate hydrogels leads to a lack of cell adhesion and, as a consequence, to an impairment of cellular responsiveness. To overcome these drawbacks of alginate hydrogels, different approaches have been studied, such as surface functionalization or the combination with other natural or synthetic polymers with better mechanical and bioactive properties.
Polycaprolactone (PCL) is a synthetic biocompatible hydrophobic polymer with excellent mechanical properties widely used in different tissue engineering applications and drug delivery [5,6]. Therefore, in this study we have focused on the combination of PCL with calcium-crosslinked sodium alginate (SA) as a promising approach to develop a semi-interpenetrated polymer network (semi-IPN) hydrogel with combined properties and wider range of applications in the biomedical field. In addition, to provide this novel hybrid system with electrical conductive properties to be employed in electroactive tissues (such as musculoskeletal, neural, bone or cardiac), two different concentrations of reduced graphene oxide (rGO 0,5% and 2% wt/wt) were embedded within the polymeric matrix. rGO is a graphene-based material (GBM) with remarkable conductive and mechanical properties which have been previously used regenerative medicine as filler to provide bioactivity in the form of electric conductivity [7-9].
The results show that the SA/PCL/rGO semi-IPN possesses a homogeneous structure based on a complex nano-network with different interactions between the SA chains, rGO nanosheets and Ca^{+2} ions, while the PCL chains are distributed within the alginate network. The incorporation of rGO significantly increases the electrical conductivity of the nanohybrid hydrogels, with values in the range of muscle tissue. In vitro cultures with C2C12 murine myoblasts revealed that the conductive nanohybrid hydrogels are not cytotoxic and can greatly enhance myoblast adhesion and myogenic differentiation. These novel electroactive nanohybrid hydrogels have great potential for biomedical applications related to regeneration of electroactive tissues, particularly in skeletal muscle tissue engineering.

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keywords: nanohybrid semi-IPN, electroactivity, muscle tissue engineering, myogenic differentiation.

62825459106

ENGINEERED SURFACES FOR PARTICLE DELIVERY AND GENE SILENCING

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Nanoparticles have attracted a great deal of interest as non-viral vectors for gene delivery. However, achieving targeted delivery is difficult. Local particle delivery is achieved by exploiting the sensitivity and responsivity of cells to their mechanical environment. Particle surfaces offer the possibility to modify the properties of existing structures and determine cell behaviour through induced topographical cues. By assembling particles electrostatically, cells are only able to internalise those that they are in direct contact with; facilitating local delivery. Herein, we engineer a surface-based approach to use these physical interactions for targeted delivery of small interfering RNA (siRNA) to suppress expression of green fluorescent protein (GFP). Silica particles (500 nm) are used as a templating core for siRNA-based polyelectrolyte multilayers. These particles are then coated with hyaluronic acid and electrostatically adsorbed to a surface using poly(L-lysine). We demonstrate that A549 remove particles from the surface with focussed ion beam imaging (FIB-SEM). Culture of GFP-expressing A549 on siRNA particle surfaces led to subsequent particle removal, internalisation and GFP-knockdown. This represents a powerful approach for designing biomaterial surfaces with local bio-instructive behaviour.

keywords: gene delivery, particle uptake, surface engineering

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GELATIN IMMOBILIZATION ON ELECTROSPUN ALIPHATIC POLYESTER FIBERS FOR TISSUE ENGINEERING

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Introduction: Immobilization of cell adhesive proteins on the scaffold surface has become a widely reported method for improving the interaction between scaffold and cells [1]. Immobilization is performed using various methods based on chemical binding or physical interaction between biomolecules and polymer. Benefits, as well as weak points of both ways, are under discussion in the literature [2].

Methodology: In this study, three types of nanofibrous nonwoven material obtained by solution electrospinning - poly(caprolactone) (PCL), poly(L-lactide-co-caprolactone) (PLCL) 70:30, and poly(L-lactide) (PLLA) were subjected to chemical immobilization of gelatin, preceded by aminolysis and glutaraldehyde cross-linking. For a comparison, a method of direct physisorption of gelatin was also applied.

Results: It was shown that the concentration of gelatin on the material's surface depends on free amine groups concentration, being the lowest for zero amine groups concentration, i.e., for physisorption. The physisorption of gelatin is unexpectedly high, most probably as a result of various molecular interactions including ionic and hydrophobic interactions as well as interactions with carboxyl and hydroxyl groups created on the polyester surface by spontaneous hydrolysis in an aqueous medium during gelatin immobilization. However, it is observed that the gelatin layers adsorbed physically are relatively unstable compared to those adsorbed chemically after the preceding aminolysis. An additional increase of gelatin content above the physisorbed level can be achieved by aminolysis leading to a non-zero surface concentration of amines. It is evident for this kind of immobilization that the sensitivity of gelatin immobilization on amine concentration is higher for PCL than for PLLA and PLCL. The highest level of efficiency of both physical and chemical gelatin immobilization results in the highest content of immobilized gelatin on PCL fibers. XPS analysis confirmed that gelatin concentration is higher for the chemically modified samples. On the basis of XPS results for PCL, the thickness of both physically and chemically immobilized gelatin layers was estimated to be less than 10 nm. For all gelatin-coated samples, complete wettability was observed, and the time of water drop absorption into the nonwoven was correlated with the surface concentration of gelatin. Immobilization of gelatin, both through physical and chemical interactions, results in improved L929 cell attachment and spreading indicating a positive binding effect of gelatin. In the case of PCL, gradual increase in metabolic activity with gelatin content was observed. For the other two polymers, no clear correlation was observed, however, all nonwovens after modification were biocompatible.

Conclusions: It was shown that chemical immobilization could provide a higher concentration

of gelatin on the fiber surface and the coating is more stable than in the case of physisorption. L929 cells imaging and results of metabolic activity tests indicate a positive effect of both physical and chemical modification on cell-scaffold interaction, whose intensity depends on the type of polymer fibers.

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keywords: surface modification, nanofibers, aminolysis, gelatin

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GELLAN GUM-GELATIN HYDROGELS ENZYMATICALLY OR CHEMICALLY MODIFIED BY CONTACT WITH POLY(VINYL ALCOHOL) BLENDS FOR SACRIFICIAL 3D PRINTING IN BONE AND CARTILAGE REGENERATION

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Introduction
Poly(vinyl alcohol) (PVA) may be used in 3D printing as a sacrificial material in the formation of tissue engineering constructs with complicated architectures. Our research aims to combine 3D printing scaffold fabrication based on PVA as a sacrificial material with enzymatic mineralization and chemical crosslinking of hydrogels, which are done by incorporating specific chemicals into PVA filament. The method to obtain the scaffold involves pouring gelan gum-gelatin (GG-Gel) hydrogel into PVA 3D printed molds. A 3D printed scaffold, being a selectively mineralized and cross-linked hydrogel, can potentially be used in bone and cartilage regeneration. In this study, as a proof of concept, we established a 2D simplified setup of the molding process to assess the ongoing enzymatic mineralization and chemical crosslinking of hydrogels.

Methodology
PVA (Mowiol, 4-88) was formed into 0.2 mm foils containing calcium glycerophosphate (CaGP) or tannic acid (TA) as a substitute for 3D printed mold material. The hydrogel containing defined amounts of gellan gum (GG), gelatin, CaCl2, and alkaline phosphatase (ALP) was poured on PVA enclosed in a rectangular container. The mineralization of the hydrogels in CaGP or chemical crosslinking with TA were performed over 1 day. Enzymatic mineralization involved the activity of ALP incorporated in the hydrogel cases and the release of inorganic phosphate from CaGP. Chemical crosslinking was obtained by reaction between the amine groups of gelatin and TA. The modified hydrogel strips were washed in water. Hydrogels as well as PVA blends were analysed under light microscopy and FTIR. The percentage of dry mass percentage of hydrogels was measured. Biological properties were tested by culture of MG-63 cells on materials for 3 days. Alamar blue and live-dead (calcein AM, propidium iodide) staining tests were performed.

Results
CaGP-containing PVA foils were translucent and mostly homogenic, although they contained gas bubbles, while TA-PVA was completely homogenic and transparent with an orange tint. GG-Gel hydrogels in contact with PVA foils underwent chemical and physical modifications. In the case of the TA-PVA, the whole mass of material changed color to bronze, and the material was homogeneous with rough, wavy surface. The CaGP-PVA-modified materials were opaque with small crystalline precipitates on the surface. Mineralized was only part of the hydrogel in contact with PVA (0.5 mm depth). FTIR spectra confirmed presence of TA phenol groups as well as PO43–. TA-modified GG-Gel material had the largest cell growth supporting ability compared to the unmodified control. For enzymatically mineralized material, increased cell spreading and attachment was also observed, but this effect was smaller.

Conclusion
Enzymatic mineralization by ALP and CaGP as well as chemical cross-linking by TA can be obtained by incorporation of desired chemicals into potentially 3D-printable PVA. Modifications significantly improved the attachment and viability of MG-63 bone cells. However, chemical cross-linking of GG-Gel resulted in overall better morphology and cell attachment.

Unfortunately, the method combining enzymatic mineralization with TA-crosslinking was not possible so far because of the inactivation of ALP by TA.

This study was supported by the National Science Centre Poland (No 2018/29/N/ST8/01544).

keywords: bone, hydrogels, mineralization

31412728077

GUIDING HUMAN-MUSCLE DERIVED STEM CELL DIFFERENTIATION TOWARDS CHONDROCYTES BY HYDROGEL SCAFFOLDS RELEASING GROWTH FACTORS

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Cartilage is a non-vascular connective tissue composed of specialized cells – chondrocytes. The lack of blood vessels results in limited cartilage self-regeneration capabilities. While various options are available to treat osteoarthritis, no current method can restore the mechanical function of the original hyaline cartilage. Therefore, herein we address, hyaline cartilage regeneration issue by engineering a synthetic biocompatible hydrogel scaffold capable to promote chondrogenic differentiation.

In this study, the chemically crosslinked hydrogels consisting of synthetic peptides that have the collagen-like sequence Cys-Gly-(Pro-Lys-Gly)4 (Pro-Hyp-Gly)4 (Asp-Hyp-Gly)4- conjugated with the cell adhesion sequence RGD (CLP-RGD) and crosslinked hydrogels of type I collagen (CA) were used. Both hydrogels were compared as cell growth substrates potentially applicable as implantable biomaterials for cartilage regeneration. For cartilage formation, we used human skeletal muscle-derived stem/progenitor cells (hMDSPCs) set for differentiation towards a chondrogenic lineage by BMP-7 and TGF-ß3 growth factors. Since the precise amount at which the growth factors induce differentiation is unknown, we chose to investigate minimal growth factors dosages, relying on the ability of the two types of hydrogels to retain and release the growth factors into cell culture media. We monitored the amount of growth factors diffusing out of the scaffolds for two weeks by changing the cell culture media and observed almost identical diffusion of the different amount of initially loaded growth factors (150, 100 and 75 ng/scaffold). Growth factors incorporation strategy allowed a sustained release of TGFß3, 6.0 0.3% of the initially loaded amount diffused out after 4 h and 2.7 0.5% already at the second time point (24h) from CA and CLP-RGD substrates. For the BMP-7 growth factor, 13.1 2.3% and 15.75 1.6% of the initially loaded amount diffused out after 4 h, 1.7 0.2% and 2.45 0.3% at the second time point (24 h) from CA and CLP-RGD respectively. We monitored growth factors diffusion out during all incubation period (14 days). In vitro experiments were performed by seeding hMDSPCs onto hydrogels scaffolds loaded with growth factors (75ng/scaffold) and cultured for 28 days. Cartilage formation was monitored by extracellular matrix protein collagen type II deposition (using biochemical ELISA assay) and quantification of glycosaminoglycans genes expression (RT-PCR). We measured collagen type II expression at different time points for 28 days. From CLP-RGD scaffolds onwards, collagen type II was significantly higher expressed than on CA hydrogel irrespective of growth factors present. The scaffolds with immobilized growth factors resulted in higher collagen type II accumulation when compared to the scaffolds alone. The gene expression on CLP-RGD hydrogels (day 28) with growth factors has shown lower collagen type I expression and higher aggrecan expression compared to day 0. However, we also report increased collagen X gene expression on CA hydrogels (with growth factors) that can be expected considering hydrogel scaffold composition which can lead to chondrocytes hypertrophy.
Our results support the potential of the strategy of combining implantable hydrogels functionalized with differentiation factors toward improving cartilage repair via precision tissue engineering.

keywords: Hydrogels, growth factors, chondrogenic differentiation

62825438705

HEAT AND PRESSURE SOFT LITHOGRAPHY-ASSISTED MULTISCALE SCAFFOLDS FOR SOFT AND HARD TISSUE REGENERATION

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Here, we present an analysis of nanoscale deformation of PDMS molds in response to heat and pressure during the repetitive molding process of thermoplastic polymers. The width and height of the nano-sized ridges of PDMS molds decreased as the number of replications of thermoplastic polymers increased. Using the precisely controlled deformation of nanostructures in PDMS molds, we demonstrated that nanostructures of different sizes can be fabricated on representative thermoplastic and UV-curable polymers consistently. Using the precisely tunable methodologies of nanoscale structures, we propose a methodology to fabricate hierarchical multiscale scaffolds with controlled hydrophilic and hydrophobic properties by employing capillary force lithography in combination with oxygen plasma modification. In response to multiscale nanotopographic and chemically modified surface cues, the O-FMN patch enhanced regeneration of the mineralized fibrocartilage tissue of the tendon-bone interface and the calvarial bone tissue in vivo in rat models.

keywords: Bioinspired scaffold, Multiscale topography, Nanotopograpy, Plasma modification, Tissue engineering

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IMMOBILIZATION OF THE ANTIMICROBIAL PEPTIDE MELIMINE ON MEDICAL-GRADE POLYCAPROLACTONE SCAFFOLDS FOR THE PREVENTION OF BIOMATERIAL-RELATED INFECTIONS

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Introduction
Implant related infections result from persistent bacteria adhering to the biomaterial surface before, during, or after surgery, enabling colonization and biofilm formation on the implant [1]. On average, 4% to 10% of implant surfaces are estimated to be contaminated with bacteria, however, the infection rate can be as high as 30% in intensive care units in developed countries, and as high as 45% in developing countries [2].
Antimicrobial peptides (AMPs) have emerged as a promising alternative to combat a broad spectrum of multidrug-resistant and persistent bacteria as they have shown to be able to successfully prevent bacteria adhesion to biomaterials, to kill bacteria residing within biofilms and to rupture the biofilm structure. Notably, recent studies have shown that covalent immobilization of AMPs onto different biomaterial surfaces increase their long-term stability in vivo and offer a proper orientation of the peptide that may result in enhanced antimicrobial activity [3]. In this study, melimine, a chimeric cationic peptide that has been tested in Phase I and II/III human clinical trials, is covalently immobilized onto the surface of 3D printed medical-grade polycaprolactone (mPCL) scaffolds. The ability of melimine-tethered surfaces to inhibit S. aureus and P. aeruginosa bacteria adhesion is assessed, as well as their ability to prevent biofilm formation in vitro.

Methodology
Macroporous 3D printed mPCL scaffolds were surface treated using a vacuum plasma cleaner (PDC-002-HP Harrick Plasma, USA) under O2/Ar2 for 6 min at high (45W) power. Plasma-treated scaffolds were then incubated in 2mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in acetate buffer for 30 minutes at room temperature. Scaffolds were washed twice with PBS pH 7.4, and subsequently incubated with a solution of 2 mg/ml melimine for 5 hours to allow covalent binding. Treated surfaces were characterized by X-ray photoelectron spectroscopy (XPS), quartz crystal microbalance (QCM) and time of flight secondary ion mass spectrometry (ToF-SIMS). Furthermore, 3D in vitro assays were used in order to investigate the antibacterial effectiveness of treated scaffolds against S. aureus and P. aeruginosa.

Results
XPS and ToF-SIMS spectra of melimine-treated surfaces confirmed the covalent immobilization of the peptide, as well as its homogeneous distribution throughout the scaffold surface. Further surface characterization using amino acid analysis showed that 82.2 ± 12.4ng of melimine were immobilized onto each scaffold. In addition, the presence of melimine on the surface resulted in a reduction of Staphylococcus aureus and Pseudomonas aeruginosa colonization by 78.4% and 74.1%, respectively, in comparison to the non-modified control specimens. Surfaces maintained their antibacterial properties for 3 days, evidencing inhibition of biofilm formation in vitro.

Conclusion
The results of this study showed the in vitro efficacy of the melimine-treated mPCL surfaces against bacterial colonization.

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keywords: Implant Related Infection, Antimicrobial Peptide, Polycaprolactone Scaffold

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MICRO-MUSCLE-WIRE FOR BIOACTUATOR

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Purpose/Objectives: Bioactuator is a structure that responds to external stimuli by combining an artificial flexible structure and a living biosystem (cells and tissues). Here, we generated and experimented with a structure whose movement can be controlled by external signals by attaching myoblasts to an elastic material in the form of a micro-wire for a bioactuator.

Methodology: Micro-wire with three or six hundred micrometer diameter was generated using various ratios of curing agent with polydimethylsiloxane (PDMS). The generated micro-wire was characterized by studying the stiffness, strength, and then myoblasts were adhered and cultured onto the convex surface after surface treatment. The cell-attached microwire was cultured with electrical stimulation for 7 days after the differentiation period.

Results: It was confirmed that myoblasts adhered onto the convex surface of the micro-wire until the end of the experiment. The myofibers were observed with the microwire and aligned morphology. The skeletal muscle-based microwire was showed a higher movement with electrical stimulation in a group of electrical cultures.

Conclusion/Significance: Thus, it has been shown that a simple micro-wire is implemented by electrical stimulation for biohybrid actuator.

keywords: Micro-wire, Bioactuator, Electrical stimulation

94238152929

PEOT/PBT ELECTROSPUN SCAFFOLDS TARGETING OSTEOPOROSIS

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Introduction
Osteoporosis is a disease that interferes with bone homeostasis making the bone weak and susceptible to fractures. One of the areas affected is the pelvis, whose fractures force the patients to undergo invasive surgeries and long bed recovery.
Here, we aim at fabricating an electrospun composite scaffold based on PEOT/PBT and loaded with particles to promote bone regeneration as well as bioactive molecules to target osteoporosis. Mesoporous bioactive glasses (MBGs) and hydroxyapatite (nanoHA) particles were included due to their high bioactive character and ability to mimic bone morphological and chemical features. To hinder the osteoporotic process, ICOS-Fc, a molecule able to reversibly inhibit osteoclast activity, was grafted on the MBGs or directly on the PEOT/PBT scaffolds.

Methodology
Electrospun PEOT/PBT-based composite scaffolds were fabricated by dissolving 28% PEOT/PBT in chloroform:hexafluoroisopropanol (CHCl3:HFIP). MBGs and nanoHA particles were then incorporated at concentrations ranging from 5% to 15%. Composite scaffolds were characterized by means of scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and energy-dispersive X-ray spectroscopy (EDX).
Afterwards, MBGs were functionalized with ICOS-Fc (MBG_ICOS)1. MBG_ICOS were sonicated and afterwards incubated in CHCl3:HFIP. Supernatants were collected after sonication and incubation steps and particles were left to air dry for complete solvent removal. An ELISA-like assay was used to determine the presence and activity of ICOS-Fc in the supernatant and on the MBG_ICOS.
Alternatively, to graft ICOS-Fc directly onto the surface, scaffolds underwent aminolysis and then EDC/NHS chemistry. The efficiency of the grafting was evaluated through an ELISA-like assay and fluorescence using a fluorescent ICOS-Fc ligand (ICOS-L).
Finally, the effect of the ICOS-Fc on the scaffolds will be assessed by migration on U2OS, a human osteosarcoma cell line, positive for ICOSL.

Results
PEOT/PBT scaffolds were electrospun with MBGs and nanoHA at a percentage of up to 12,5% and 15%, respectively. Composite scaffolds showed a uniform distribution of particles into the

meshes.
MBG_ICOS showed to be stable in the CHCl3:HFIP electrospinning solvent. In particular, the functionalized particles showed no decrease of presence and activity of ICOS-Fc before and after incubation in the solvent. Additionally, the supernatant collected after the sonication and incubation steps showed no ICOS-Fc present.
To graft ICOS-Fc on the meshes, aminolysis and EDC/NHS chemistry showed the possibility to bind around 25 ug/ml of ICOS-Fc to the scaffolds, which was measured through an ELISA-like assay. ICOS-L further confirmed the presence of the molecule on the fibers.
It is expected that the mobility of U2OS cells will be inhibited on the scaffolds, therefore evidencing the effect of ICOS-Fc on cell migration.

Conclusions
In conclusion, PEOT/PBT scaffolds were successfully spun alone and with MBGs and nanoHA particles. In addition, ICOS-Fc was incorporated into the meshes by functionalizing MBGs or by directly grafting on the electrospun fibers.
These results showed the potential to include in electrospun scaffolds not only particles such as nanoHA and MBGs, but also bioactive molecules able to target osteoporosis.

1. Fiorilli, S., et al., Nanomaterials (Basel) (2021) 11 (2), 321

keywords: Electrospinning, Osteoporosis, Bone

83767219746

SURFACE STIFFNESS DEPENDENT GINGIVAL MESENCHYMAL STEM CELL SENSITIVITY TO OXIDATIVE STRESS

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Introduction
Mesenchymal stem cells (MSCs) are widely used in the fields of cell therapy and tissue engineering, due to their wide spectrum of differentiation potential, immunomodulation function and ongoing oxidative stress (OS) reduction. OS impact is often overlooked in these research fields, even though, it is not only responsible for the induction and development of many ailments, e.g., diabetes, lung fibrosis, and cancer, but it also causes stem cell death and senescence during cell therapy and tissue engineering practices. As MSCs are used to treat different types of tissues, these cells interact with different tissue-specific mechanical environments, thus it is important to understand how the mechanical environment impacts MSC sensitivity to OS.

Methodology
In this study, human gingival mesenchymal stem cells (GMSCs) were used (approval No. 158200-16- 860-369 2016-07-12) To study the impact of mechanical environment collagen type I coated acrylamide - bisacrylamide hydrogels were used. Three different stiffness surfaces, which mimicked soft (1 kPa), medium (8 kPa) and hardest (40 kPa) stiffness tissues, were used in this study. Cell-surface interaction was observed with immunocytochemistry, proliferation and cytotoxicity evaluation methods. Mitogen-activated protein kinases (MAPK) – ERK, JNK, p38 and transcription factors c-Jun and c-Fos role was evaluated by Western blot and MAPK inhibitors assays.

Results
Gingival MSCs were isolated from healthy volunteers during wisdom tooth extraction. These cells expressed CD44, CD90, CD105, CD166 surface markers, were negative for hematopoietic CD19, CD45 markers, and were able to differentiate into adipose, muscle and osteogenic cells. GMSCs interaction with polyacrylamide hydrogels were surface stiffness depended. The stiffer the surface the more strongly cells interacted with the hydrogels. Also, cells demonstrated higher proliferative activity on stiffer surfaces. Furthermore, GMSC sensitivity to OS increased on softer surfaces. MAPK expression analysis revealed, that their levels and phosphorylation is also surface stiffness dependent. Even so, their role changes when GMSC were grown on different stiffness polyacrylamide hydrogels.

Conclusions
In this work, for the first time, as known to the authors, it was shown that GMSCs sensitivity to OS depends on the stiffness of the surface, on which the cells are grown. Furthermore, the activity and expression of MAPK ERK, JNK, and p38 were also surface stiffness dependent. GMSCs isolated from intermediate/stiff gingiva tissue (~20 kPa) have shown the best proliferative

and survival properties, then grown on the stiffest tissues mimicking polyacrylamide hydrogels (40 kPa). Therefore, MSC source might determine their sensitivity to OS in different stiffness environments and should be accounted for when developing a treatment strategy.

keywords: Mechanobiology, Gingival MSC, Oxydative stress, MAPK, AP-1

73296306605

TEMPERATURE EFFECT ON PHYSIOCHEMICAL AND BIOLOGICAL PROPERTIES OF CROSS-LINKED PNIPAM-GRAFTED-CHITOSAN/HEPARIN MULTILAYER

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The lack of bioactivity of poly(N-isopropylacrylamide) (PNIPAM) restricts the use in cell-containing systems to the support as thermoresponsive adhesive substratum with no effect on cell differentiation. In this study, layer-by-layer assembly of polyelectrolytes, allows the combination of PNIPAM-grafted-chitosan (PNIPAM-Chi) with bioactive heparin (Hep) to fabricate PNIPAM−modified polyelectrolyte multilayers (PNIPAM−PEM). Owing to lower critical solution temperature of PNIPAM at around 32 °C, the physiochemical and biological properties of PNIPAM−PEM can be thermally modulated.
The thermorepsonsive behaviors of PNIPAM-Chi with different sizes and degree of substitution (DS) are ensured by dynamic laser scattering. The PNIPAM-PEMs are formed from selected PNIPAM-Chi derivatives as polycation and Hep as polyanion at pH 4. Subsequently, chemical cross-linking is introduced to stabilize the PEM. The temperature effects on wetting properties roughness and stiffness of the PNIPAM−PEMs with either PNIPAM-Chi− or Hep− terminal layer are investigated by different analytical methods. In addition, the stability of the PEMs is tested by rinsing with PBS, pH 7.4 and DMEM. Furthermore, the association of adhesive protein vitronectin with PNIPAM-PEMs at 20 and 37 °C is studied for their biological function. The application of PNIPAM-PEMs as cell culture substrate is finally examined using multipotent mouse stem cells in the presence of vitronectin.
PNIPAM-Chi with either higher DS or size showing strong thermoresponsiveness across LCST are selected to from multilayer with heparin. Ellipsometry results confirm that irreversible cross-linking retains the layer integrity after exposure to physiological buffer at pH 7.4 compared to those without cross-linking. Upon temperature change, PNIPAM-PEMs particularly Hep as terminal layer not only exhibit smoother and more hydrophobic surface at 37 °C than at 20 °C but also are stiffer than those PEMs without PNIPAM. This might also take account of higher retention of vitronectin on Hep− terminated at 37 °C. Finally, with the aid of pre-adsorbed vitronectin, cell adhesion and spreading are improved on both PNIPAM-Chi− and Hep− terminated layers.
In conclusion, the amide bonds make an irreversible bond formation between PNIPAM-Chi and Hep to enhance stability of PEM. In addition, the PNIAPM immobilized on the surface affects the wetting and mechanical properties of the surfaces. Therefore, the PNIPAM-PEMs with cross-linking provides greater stability and biological function as a cell culture system to potentially promotes tissue regeneration.

keywords: Polyelectrolyte multilayer, Heparin, PINPAM-grafted-chitosan, Temperature, Vitronectin

52354522724

THE EFFECT OF VASCULAR PROSTHESES SURFACE MODIFICATION WITH REDV AND YIGSR PEPTIDES ON HEMO- AND BIOCOMPATIBILITY

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The usage of artificial materials for tissue engineering is well-known. Their use as a vascular prosthesis is being considered more often. One of the most promising materials for use for applications in the cardiovascular system is polyurethane which has a good bio- and hemocompatibility as well as appropriate mechanical properties. However, researchers are still looking for methods to improve it in order to reduce the risk of an unwanted reaction of the human body. The most promising approach is seeding vascular graft with endothelial cells.
The effect of surface modification with REDV and YIGSR peptides on hemocompatibility and competitive adhesion of endothelial and smooth muscle cells was examined. According to the literature, these sequences are supposed to promote endothelial cell adhesion, while not significantly affects the modified material hemocompatibility. Several methods of endothelial cells seeding on the inner surface of cylindrical grafts were investigated. In the next stage of the research, the coculture of endothelial cells and smooth muscle cells was carried out. The last stage of the study was the analysis of the interaction of the tested materials with blood under flow conditions. The number and morphology of the platelets adhering to the surface of the prosthesis were analyzed, as well as their activity (CD62P + platelets).
The analysis of the co-culture results showed that the presence of REDV and YIGSR peptides promoted selective adhesion of endothelial cells, and investigation of the blood-material interaction showed a reduction in the percentage of platelets area on the surface of the peptide-modified prostheses.

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keywords: polyurethane, REDV, YIGSR, hemocompatibility, vascular prostheses

94238108524

ULTRATHIN COATINGS OF EXTRACELLULAR MATRIX-MIMETIC PEPTIDE HYDROGELS FOR CONTROLLED CELL ADHESION AND TISSUE FORMATION

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We have studied synthesis and properties of ultrathin hydrogel coatings with a synthetic extracellular matrix (ECM) analogue: a chimeric peptide comprising a collagen-like peptide block Cys-Gly-(Pro-Lys-Gly)4 (Pro-Hyp-Gly)4 (Asp-Hyp-Gly)4- and the fibronectin RGD block -Arg-Gly-Asp-Ser-Pro-Gly (hereafter CLP-RGD) [1]. For ultrathin hydrogel functionalized with the peptide, we employed initiator free, UV-controlled self-initiated photografting and photopolymerization reaction, on two different substrates: on glass [2] and medical grade PMMA plastic. In first step, the CLP-RGD peptide was functionalized via Michael addition reaction with methacrylate group using the 3-(acryloyloxy)-2-hydroxypropyl methacrylate reagent, thus obtaining a meathacrylate(MA)-Cys-CLP-RGD functional peptide. By combining MA-Cys-CLP-RGD, 2-hydroxyethyl methacrylate, PEG methacrylate and methacrylic acid monomers in self-initiated photografting and photopolymerization reaction we successfully synthesised 15-50 nm thick, mechanically stable ECM-mimetic peptide hydrogels. The hydrogel coatings were seeded with human skin fibroblast and human cornea epithelium cell lines, respectively. Both cell types formed monolayers on the hydrogel within 96 hours. Our study shows the potential of the fully synthetic ECM-like coatings for bioanalytical, tissue engineering and regenerative medicine application, especially applicable in the high throughput and biochip formats.

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keywords: Synthetic ECM, peptide, hydrogel, thin, coating

PS14
Biological testing of 3D-printed biomaterials – towards updated norms

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ADDITIVE MANUFACTURING OF OSTEOINDUCTIVE SCAFFOLDS USING CALCIUM PHOSPHATE: EXTRUSION-BASED PRINTING AND DIGITAL LIGHT PROCESSING TECHNOLOGIES

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Introduction:
Critical-sized bone defects can result from trauma, inflammation, and tumor resection. Such bone defects, often have irregular shapes, resulting in the need for new technologies to produce suitable implants. Bioprinting is an additive manufacturing method to create complex and individualized bone constructs, which can already include vital cells.
In this study, we present extrusion-based printing for the creation of a mechanically stable outer ring with the shape of a cylinder. The digital light processing technology was applied to produce a biologized inner core, which fits into the outer ring. Both scaffolds contained calcium phosphate, which is known to induce osteogenic differentiation of stem cells.
Methodology:
The model with the spongiosa-like structure was created in python based on the signed distance functions. The inner construct (diameter: 3.5 mm, height: 10 mm) and the outer construct in form of a ring (diameter: 20 mm, height: 10 mm) have an irregular, interconnected porous structure with a diameter of 2 mm +/- 0.2 mm standard deviation, which mimic the natural spongiosa structure of bone.
For digital light processing printing technology, the Lumen X (CELLINK, Holecombe, USA) was used. The Photoink polyethylene glycol diacrylate (PEGDA) was modified with calcium phosphate nanopowder (> 150 nm particle size). Before printing, human mesenchymal stem cells (hMSC) (3 x 106 cells/ml) were encapsulated into the bioink. During the layer-by-layer printing process, the bioink was exposed to ultraviolet light (405 nm) to initiate polymerization.
Extrusion-based printing was conducted using the BIO X6 (CELLINK). Polycaprolactone (PCL) (80 kDa) was combined under heating with calcium phosphate nanopowder in a ratio 1:8 (w/w). After printing, 5 x 106 hMSC were seeded on the construct with the help of a rotation incubator.
The viability of encapsulated cells was examined with a live/dead staining using calceinAm and propidium iodide, respectively.
Results:
Digital light processing: The encapsulated hMSC inside the printed construct of PEGDA-calcium phosphate construct showed a typical elongated morphology and partly formed cell clusters. The life/dead staining revealed, that hMSC were vital over a time span of 22 days in the printed PEGDA construct.
Extrusion-based printing: We were able to print a highly accurate ring construct with an interconnected pore structure.The PCL combined with calcium phosphate particles resulted in a precise printed construct, which corresponded to the 3D model. The bioink containing calcium phosphate nanoparticles had a higher printing accuracy compared to PCL alone. We found that hMSC cultured on the construct settled in close proximity to the calcium phosphate particles. The hMSC were vital for 22 days on the construct as demonstrated by life/dead staining.

Conclusion(s):
With both printing technologies, it was possible to print spongiosa-like structures. The PCL scaffold offered sufficiently strong mechanics similar to bone. To improve the biological properties of the scaffold, a soft spongiosa-like structure printed with PEGDA could serve as an inner filling. Composite materials mimic bone tissue better than one of the presented materials alone and therefore represents a promising option for the use in regenerative medicine.

keywords: Bioprinting, Bone repair, Implant, Additive Manufacturing

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CELL GROWTH MECHANICS IN GELATIN/ALGINATE BASED HYDROGELS

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While both, regenerative medicine and tissue engineering shift from 2D based models towards 3D models, problems related to imaging of those difficult constructs became more relevant than ever. One of the most attractive technics used by the aforementioned fields is 3D bioprinting based on hydrogel scaffolds. Unfortunately, due to the complexity of the growth kinetic of cells in the printed hydrogels imaging choices and approaches can vary, especially due to multiple morphological architypes of cell growth.
We have tested multiple Gelatin/Alginate based hydrogels as a growth scaffold for two cell lines with different growth kinetics to determine the tendencies of scaffold population and morphological preferences of tested cells. We have also determined best imaging practices for such hydrogels, scaffolds maintaining sterile conditions.
Mouse Fibroblasts (3T3 cell line) and Human Keratinocytes (KerCT cell line) were grown in hydrogels for a maximum of 4 weeks and observations of key growth events were performed both with live imaging and fixated sample imaging using fluorescence technics. Gel biocompatibility was tested using custom Calcein AM staining. Observations of the residual cell layer post- hydrogel transfer was also performed during the experiment.
Hydrogels containing 6% Gelatin and 2% Alginate used in this study were based on the, recommended growth medium: DMEM/F12K for 3T3 and KBM Gold for KerCT cell line. After adding DAS (Dialdehyde starch) / SQ (Squaric acid) 0,5 milion cells / ml of gel were added and mixed. The final step was crosslinking the hydrogels with calcium chloride and washing. Hydrogels were incubated in a cell growth CO2 controlled incubator with the dedicated growth medium and the media was replaced every 72hours. When excessive cell growth was observed on the bottom of the culture vessel, hydrogel scaffolds were migrated to a fresh vessel.
Both hydrogel types were found biocompatible with the tested cell lines. We have observed a crucial relationship between both, growth kinetics and the morphological architypes of cells depending on the hydrogel thickness and degradation level. The 3T3 cell line favored rapid growth in damaged areas of hydrogels where the observed morphology was most comoplex. KerCT cells did also exhibit faster growth in the same areas, but due to the naturally slower growth kinetics it was less visible. Cell growth observed within the thicker areas of gels (above 200 μm) was considerably slower, and occurred after partial gel degradation nearing the third week of cell culture. For both cell lines spheroid formation was occurring near the peripheral areas, but was more common for hydrogels crosslinked using SQ.
The research was funded by NCBiR (MATURO 3D project) and from internal CM UMK idub equipment grant.

keywords: biomaterials, hydrogels, spheroid, imaging

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THE POTENTIAL OF MULBERRY AND NON-MULBERRY SILK FIBROIN BLENDS AS BIOINKS FOR MENISCUS REGENERATION BY 3D-BIOPRINTING

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INTRODUCTION
The meniscus is vital for biomechanical and anatomical purposes in the knee, which is frequently injured. Among the diverse biomaterials used for 3D-bioprinting in meniscus regeneration research, silk fibroin (SF) derived from mulberry Bombyx mori (BM) silkworm is extensively studied. Its main drawbacks are the challenge to print it without another material and its lack of the Arg-Gly-Asp (RGD) sequence for cell adhesion. Therefore, non-mulberry silk types like the one from Antheraea mylitta (AM), with the inherent RGD motif, are also being explored. Thus, our approach of blending mulberry and non-mulberry silk fibroin provides a new approach to obtain a printable hydrogel for meniscus regeneration.

METHODOLOGY
Bioink preparation: The cocoons were cut and degummed. BM SF fibers were dissolved and the solutions were dialysed against water for 2 days with regular water exchanges.
Gelation kinetics: BM and AM SF solutions were mixed in different proportions without any crosslinker and diluted to yield blends with 3-10% (w/v) SF. The absorbance at 540 nm was monitored for 3 h at 37°C in a plate reader.
Printability: BM and AM SF solutions were mixed (1:1) to yield 5% and 10% (w/v) SF. Solutions with/without ruthenium photoinitiator kit were prepared. After gelation in syringes at 37°C the gels were extruded into the shape of a meniscus on a bioprinter with/without visible light for crosslinking.
Cell viability: Chondrocytes were seeded into 10% (w/v) BM SF and SF blend with ruthenium photoinitiator. The gels were incubated at 37°C for 3 days, before staining with a Viability/ Cytotoxicity Kit.
SEM: The gels for cell viability evaluation were iamged with FlexSEM 1000.

RESULTS
Gelation kinetics: The addition of more AM SF than BM SF has proven to be advantageous for the gelation time. Only when three times as much AM SF was added, the gelation time increased again.
Printability: Printing 5% (w/v) SF blends and already crosslinked and non-crosslinked 10% (w/v) SF blend resulted in unstable constructs. Therefore, the 10% (w/v) SF blend with crosslinking during printing has proven to be the best condition for printing a meniscus.
Cell viability: In the BM SF hydrogel, only a few live and dead cells were detected. In the SF blend hydrogels, no dead cells and a higher number of living cells were found.
SEM: The combination of BM SF and AM SF led to a higher pore size number than the use of BM SF alone. The pores made up approx. 25% of the total area.

CONCLUSION
Our study has shown that the BM and AM SF blend is suitable as a novel bioink, since it possesses self-gelation properties. With the appropriate proportion of SF, the solution gels

within 90 min and can be applied as bioink with photocrosslinking during extrusion on a bioprinter. Our first results regarding printability and property analysis of SF blend hydrogels show promising potential for the application as bioink. Additionally, the initial cell viability studies showed that the blend constitutes a better environment for cell proliferation and survival than BM SF alone.

keywords: bioprinting, meniscus, silk

PS15
Biologically inspired and Engineered disease models

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A NEW PRINTABLE ALGINATE / HYALURONIC ACID / GELATIN HYDROGEL SUITABLE FOR BIOFABRICATION OF IN VITRO AND IN VIVO METASTATIC MELANOMA MODELS (1)

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Introduction
Tumor models have advanced over the years as scientists have made several improvements. In the past, typically 2D cultures were used. Due to their artificial nature, their results are often not transferable to the human pathophysiology. 3D models are a promising approach for a more in vivo-like microenvironment. Here, the upcoming field of biofabrication makes it possible to develop more sophisticated models with spatially arranged components, which can be used for in vitro and in vivo applications.

Methodology
With this study, we developed a printable hydrogel consisting of alginate, hyaluronic acid, and gelatin (Alg/HA/Gel) for extrusion-based bioprinting. We analyzed the bioink's printability, mechanical properties, and suitability as basis for in vitro melanoma models that mimic the tumor microenvironment. In vivo, we characterized its effect on tumor progression, vascularization, and metastasis in a defined and isolated arteriovenous (AV) loop model in the

rat.

Results
The hydrogel showed mainly elastic properties with a storage modulus of 10.5 kPa at 1 rad/s using dynamic mechanical analysis. Its stiffness was to a great extent tunable when increasing the alginate content. The bioink showed good printability and shape-fidelity. The human melanoma cell line Mel Im had high survival rates during the printing process. Cell cycle analysis with the fluorescent-based cell cycle indicator (FUCCI) of these cells did not reveal an impact on cell cycle populations over one week, neither due to the printing nor in beads. Adipose-derived stem cells were able to survive within the hydrogel and differentiate into the adipogenic and the osteogenic lineage over 21 days. This was demonstrated via Oil Red O and Alizarin red stainings and qPCR. A Gaussia princeps Luciferase fusion protein producing HEK293 cell line revealed the feasible diffusion of proteins with a size of 150 kDa through the hydrogel. In the in vivo AV loop model, the hydrogel facilitated good tumor progression, vascularization and reliable lung metastases over four weeks. This closely resembles the human pathophysiology and morphology. Whole-mount light sheet fluorescence microscopy (anti-CD31) and histological sections (HMB-45) of the explants and explanted lungs supported these findings.

Conclusion
In summary, this Alg/HA/Gel bioink is a versatile tool for basic and applied cancer research. In combination with the AV loop model, it is a unique in vivo model to study melanoma pathophysiology and possible therapies.

Reference
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keywords: tissue engineering, bioink, stem cell, tumor, metastasis

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AN IN VITRO MODEL TO STUDY THE RECOVERY OF THERAPEUTICALLY ABLATED VASCULAR NETWORKS

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Introduction
Cycles of vascular growth and regression can be observed in physiology as well as pathology. Angiogenic phases are intermitted by vessel regression in the menstrual or hair cycle and tumor-supplying vasculature can regrow after therapeutic intervention through anti-angiogenic therapies (AATs)(1-3). While AATs based on limiting the availability of VEGF have been successfully used in the clinics, it has also long been established that their efficacy varies with tumor types and can lead to development of more aggressive and heavily vascularised phenotypes upon treatment termination(3). To this day, a multitude of in vitro angiogenesis assays have been described, however, in vitro systems for modelling the recovery of a vascular network are lacking. In this study, we establish and describe a platform to generate controlled yet complex vascular networks which are sensitive to treatment with bevacizumab and which can be replenished through the addition of new endothelial cells.

Methodology
Three-dimensional cultures were grown by seeding cells onto a commercially available 96 well-plate featuring pre-cast poly(ethylene glycol)-based hydrogels (3DProSeed, Ectica Technologies). Sequentially seeded bone marrow-derived mesenchymal stem/stromal cells (BM-MSCs) and human umbilical vein endothelial cells (HUVECs, GFP expressing) were allowed to assemble into vascular-like networks for four days in presence of 50 ng/mL FGF-2. Networks were characterized by quantifying total GFP-length and staining for extracellular matrix molecules fibronectin and laminin. Bevacizumab was supplied from the start of endothelial culture to study its influence on network formation or after the four-day formation-period to study its effect on the maintenance or ablation of vascular networks. Fresh RFP-expressing HUVECs were added to pre-formed networks and their relative localization and potential recovery of regressing GFP-HUVEC-structures was monitored.

Results
After three days of BM-MSC pre-culture and four days of co-culture with GFP-HUVECs, tightly interconnected endothelial networks had formed. The two co-cultured cell types shared a basement membrane-like layer of fibronectin and laminin and could be maintained for several days of co-culture. Vascular network formation on this platform appears to be sensitive to bevacizumab, as low concentration of this compound (10 µg/mL) prevented this morphogenic process. Interestingly, pre-formed day four-networks were seen to degrade only in presence of high bevacizumab concentrations (100 µg/mL), while no change could be observed in presence of low concentrations (10 µg/mL) compared to control conditions for at least four more days of culture. When RFP-HUVECs were added to pre-formed day four-GFP networks, they were seen to integrate into the established GFP-structures and helped to extend the longevity of the total endothelial networks.

Conclusions
Our results suggest that the herein established and characterized platform can be employed to

study processes regarding both the formation and maintenance of vascular structures. Due to its easy-to-use nature, sequential seeding of cells is possible, which will be used to study if and how endothelial cells can recover an AAT-compromised or regressed vascular network.

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keywords: Angiogenesis assay, Vascular Remodelling, Antiangiogenic therapy, Co-culture model

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BIO-WASTE NATURAL BIOACTIVE COMPOUNDS AS A POWER TOOL IN ANTICANCER THERAPIES

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Introduction: Cancer is increasingly a global health issue. Each year, millions of people are diagnosed with cancer around the world and it is still a leading cause of death. Despite the significant improvement in treatment and prevention, there is a critical need for more effective strategies, like the use of synthetic or natural agents to inhibit, retard, or reverse the process of carcinogenesis. Biologically active compounds (BACs), such as polyphenols and flavonoids, are well known in nonconventional medicine due to their anti-proliferative, anti-inflammatory or anti-bacterial properties. The aim of our study was to evaluate the anti-proliferative properties of BACs, extracted from bio-waste products, on epidermoid carcinoma cells in vitro.

Methodology: Samples of the biomass were collected, dried and used for BACs extraction. After the extractions and filtration, the extracts were analysed to determine the total phenolic and flavonoids content and polyphenols composition. Anti-cancer investigations were carried out using PBS extracts containing BACs (BACs-PBS) with the use of epidermoid carcinoma cells (A-431) and mesenchymal stem cells immortalized with hTERT (ASC52telo). MTT assay was used to measure cell viability according to the ISO 10993-5:2009. Both cell lines were incubated with extracts for 24-72h. To determine cell viability, LIVE/DEAD™ Cell Imaging Kit was used. Additionaly, cells' proliferation was measured with the BrdU assay.

Results: After conventional extraction, high equilibrium values of the extracted species was observed (i.e. 24 mg/(g solid) for total phenolics and 21mg/(g solid) for total flavonoids, respectively). The ESI-MS analysis showed that the obtained extracts are mixtures of different types of phenolic compounds. The predominant one is Luteolin. After 24 h incubation, only minor changes in mitochondrial dehydrogenase activity were found. 72 h incubation with 20-fold diluted BACs-PBS extract reduced ASCs viability by 17.7 % ($p<0.01$) compared to control. Interestingly, cancer cells viability was reduced by 40.7 % ($p<0.0001$). It was noticed that the number of actively proliferating cancer cells was reduced by 25 % during the first 24 h. As measured using the thymidine analog, the total cell number decreased to 53 %. No changes were observed for ASCs. It was observed that BACs-PBS extract reduced ASCs and A-431 cells viability after 72 h by respectively 38.2 % ($p<0.01$) and 53.4 % ($p<0.001$) in comparison to control. To detect newly synthesized DNA of actively proliferating cells, BrdU assay was used. Co-staining with DAPI allowed for quantification of total cell number. It was observed that 72 h incubation with BACs-PBS extract reduced proliferation of A-431 cells by 26.7 % in comparison to control. Furthermore, cell number was reduced by 46.8 %, which could be attributed to Luteolin effect from BACs-PBS. Interestingly, no effects were observed for ASCs.

Conclusion: Based on the conducted research, we proved that BACs-PBS extract, which contains a high amount of Luteolin, induces cytotoxicity toward cancer cells, which, together with its high selectivity, robustness and nontoxicity make them very promising materials for health applications.

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keywords: bio-waste, extracts, cytotoxicity, cancer, natural materials

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DEPOSITS AND NEURO-DEGENERATIVE DISEASE: A 3D BIOPRINTED IN VITRO MODEL FOR AGE-RELATED MACULA DEGENERATION

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Introduction: Age-related macula degeneration (AMD) is a blinding neurodegenerative disease of the retina. It affects 4% of the population over 60. For over 90% of patients there is no cure due to cellular atrophy of the retina. Progressive deposit accumulation (drusen) under the retinal pigment epithelium (RPE) is the hallmark of the disease. Currently, no single (in vitro) model recapitulates this feature fully, which severely hampers pre-clinical and therapeutic research. Due to technical limitations so far, neither deposit initiation nor its consequences have been addressed in a fully controlled experimental set-up. We propose here to combine our expertise in subRPE deposit biology and our state-of-the-art 3D cellular bioprinting technology at Amsterdam UMC, to create and assess a fully controlled next-generation in vitro AMD model. This bioprinted model will consist of a monolayer of stem cell-derived RPE, a third-generation artificial Bruch's membrane (BrM) and (a combination of) single artificial subRPE deposit components in between. These deposits will contain fully controlled amounts and types of subRPE deposit material. The model will help to understand better the process of drusen progression and disease.

Methodology: The ARPE-19 cell line was used for pilot experiments. Stem cell derived neuro-epithelial (RPE) cells and endothelial cells were generated and stored. They were characterised by light and confocal scanning microscopy, PCR and by transepithelial electrical resistance measurement. The 3D deposit AMD model was printed using the RegenHU 3D Discovery bioprinter. The deposit model was characterised by light microscopy, confocal scanning microscopy. Images were analysed using ImageJ software.

Results: Embryonic stem cell differentiated RPE cells showed extensive pigmentation, expression of RPE65 and BEST1 and developed high transepithelial electrical resistance over time (602.3 ±37.3 Ohm*cm2 at day 120). Embryonic stem cell differentiated endothelial cells showed positivity for CD31 and CA4. In parallel, AMD relevant deposit material, representing drusen, was embedded in a synthetic hydrogel and 3D bioprinted in different sizes. The 3D bioprinting showed high precision and reproducibility (SD< 0.04). The electromagnetic jetting technology resulted in homogenous distribution of AMD relevant deposit material in the printed hydrogel droplets. As a next step, ARPE-19 cells were seeded on top of the 3D bioprinted deposit droplets. As documented by confocal scanning microscopy, RPE cells showed partial attachment and grew over the edge of AMD relevant deposit material contained in hydrogel droplets.
Conclusions: Ready-to-use stem cell derived RPE and endothelial cells have been established. Using cellular 3D-bioprinting technology, we showed that it is possible to recreate a

working artificial sub-RPE deposit model in a highly controlled experimental setup. Further characterization of this model is under progress.

keywords: retinal pigment epithelial cells, deposit, jetting bioprint, age-related macula degeneration

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DEVELOPMENT OF AN IN VITRO SYNTHETIC POLYMER-BASED 3D CONTRACTION MODEL FOR FIBROSIS

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Introduction:
Fibrosis contributes to 35-40 percent of deaths worldwide1. The key factors involved in fibrosis at the cellular level are increased differentiation of fibroblasts into myofibroblasts and hampered myofibroblast apoptosis, leading to persistent collagen deposition and tissue contraction2. Currently only very few drugs are clinically available for fibrosis treatment therefore there is an urgent demand for developing novel therapeutic drugs and in vitro fibrosis models to check the efficacy of these putative drugs. In this study, we developed a novel fibrosis model based on synthetic polyisocyanides (PIC-RGD) hydrogels. The model not only measures contraction but, additionally, allows for molecular and cellular analysis.

Methods
Fibroblasts were seeded in PIC-RGD gels in the absence or the presence of 10 ng/ml TGFβ1 to facilitate differentiation into myofibroblasts up to day 6. Antifibrotic drugs were added at day 6 and inhibitory effects of drug were checked at day 7.

Results/conclusions
The presence of myofibroblasts was confirmed by gene expression and immunostaining of, respectively, alpha smooth muscle actin (????SMA) and collagen 1 1 (Col1 1) in the hydrogels. Tailoring gel concentration allows for excellent discrimination between fibroblasts and myofibroblasts in terms of concentration. As proof of principle, nintedanib and pirfenidone (both are FDA-approved drugs for pulmonary fibrosis) were probed on our developed fibrosis model. The inhibiting effect of nintedanib and pirfenidone is clearly observed in reduced myofibroblast contraction. The results were confirmed by bright field imaging, immunostaining of ????SMA and live-dead staining processes.
In summary, the PIC-RGD hydrogel is highly suitable as an in vitro contraction and fibrosis platform to monitor the efficacy of various drugs and chemicals on fibrosis, scarring and molecular and cellular analyses. Our newly developed contraction and fibrosis platform offers several advantages: 1) it is synthetic and easy to modify with any desirable peptide and growth factor, 2) it has no batch to batch variations, 3) it is temperature sensitive, which allows easy isolation of cells for downstream assays and analysis such as PCR 4) no excess proteins interfere during the assays 5) the platform has virtually no autofluorescence but can easily be equipped with any fluorophore. The combination of these properties make our model an attractive candidate for high throughput screening of putative drugs against fibrosis and scarring.

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keywords: Fibrosis, hydrogels, contraction models, myofibroblastss

20941880919

ENGINEERING A 3D BONE MARROW ADIPOSE COMPOSITE TISSUE LOADING MODEL SUITABLE FOR STUDYING MECHANOBIOLOGICAL QUESTIONS

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Tissue engineering strategies are widely used to model and study the bone marrow microenvironment in healthy and pathological conditions. Yet, while bone function highly depends on mechanical stimulation, the effects of biomechanical stimuli on the bone marrow niche, specifically on bone marrow adipose tissue (BMAT) is poorly understood due to a lack of representative in vitro loading models. Here, we engineered a BMAT analog made of a GelMA (gelatin methacryloyl) hydrogel/medical-grade polycaprolactone (mPCL) scaffold composite to structurally and biologically mimic key aspects of the bone marrow microenvironment, and exploited an innovative bioreactor to study the effects of mechanical loading. Highly reproducible BMAT analogs facilitated the successful adipogenesis of human mesenchymal bone marrow stem cells. Upon long-term intermittent stimulation (1 Hz, 2 h/day, 3 days/week, 3 weeks) in the novel bioreactor, cellular proliferation and lipid accumulation were similar to unloaded controls, yet there was a significant reduction in the secretion of adipokines including leptin and adiponectin, in line with clinical evidence of reduced adipokine expression following exercise/activity. Ultimately, this innovative loading platform combined with reproducibly engineered BMAT analogs provide opportunities to study marrow physiology in greater complexity as it accounts for the dynamic mechanical microenvironment context.1

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keywords: Bioreactor, Adipose tissue model, GelMA Hydrogel, Biomechanical loading

62825421546

EXTRACELLULAR MATRIX REMODELING UPON CYCLOPHILIN INHIBITOR TREATMENT IN PATIENT DERIVED MODELS OF LIVER FIBROSIS AND INJURY

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Introduction: Cyclophilins are peptidyl-prolyl isomerases that regulate several biological processes. Importantly, cyclophilin-A promotes the remodelling of extracellular matrix (ECM) proteins by activating matrix metalloproteinases and is implicated in the correct folding of collagen fibres. Cyclophilin inhibition has shown beneficial therapeutic effects at various stages of liver disease, including steatosis, fibrosis, and hepatocellular carcinoma. As mortality caused by liver disease and its complications is on the rise, the possibility of targeting cyclophilins represents a strategy that is worth investigating. CRV431 is a pan-cyclophilin inhibitor (non-immunosuppressant cyclosporin derivative) that is currently in clinical development for non-alcoholic liver disease (Phase 2A).
Here, we developed a pipeline for evaluating the effects of CRV431 in two patient-derived models of liver disease, and in particular its role in ECM remodelling. The objectives were:
i) to study the ECM scaffold remodelling by primary human hepatic stellate cells (HSCs - major ECM-producing cells in liver fibrosis) upon fibrosis induction and treatment with CRV431;
ii) to investigate the effects of hepatotoxic insults and CRV431 treatment in a 3D-organotypic and multicellular model (patient-matched precision-cut liver slices - PCLS).
Methodology: Patient-matched PCLS and primary HSCs were prepared from human liver specimens (n=4). PCLS were exposed to hepatotoxic insults (ethanol 250mM, fatty acids 0.1mM, LPS 10µg/ml) with or without 5µM CRV431, for up to 5 days. Quiescent HSCs were activated using TGFβ1, for up to 10 days in the presence/absence of 5µM CRV431. PCLS tissue functionality was evaluated by histology, cytokeratin-18 release and mitochondrial assays. In both PCLS and HSCs, fibrosis/HSC activation status was assessed by gene expression, immunofluorescence and secretion of fibrotic markers. Pro-inflammatory cytokines in the culture media of both PCLS and HSC were quantified by Luminex. ECM remodeling was assessed via atomic force microscopy (AFM) in PCLS, while in HSC cultures, deposited ECM was decellularised and characterized via proteomics and confocal imaging. Fibre thicknesses and alignment was analysed via a Fiji-ImaJ plug-in software fit for the porpoise.

Results: Upon administration of hepatotoxic compounds in PCLS, liver damage was induced, shown by apoptosis makers and pro-fibrogenic/pro-inflammatory cytokines increase. CRV431 reduced the expression and secretion of damage-associated markers and restored a balanced cytokine profile in organotypic liver slices.
In activated HSCs, CRV431 reduced aSMA expression and collagen and fibronectin deposition when added simultaneously or after TGF-β1 activation. In addition, CRV431 treatment induced modification of ECM fibre alignment, showing that specific ECM protein remodeling is a mechanism of action of CRV431. Importantly, both in 2D and 3D models, CRV431 was not hepatotoxic and did not induce cell death.

Conclusion: Our results confirm the role of cyclophilins in liver fibrosis, including HSC activation, and ECM fibre deposition and alignment. These data reveal for the first time the potential for cyclophilin inhibitor CRV431 to reduce liver fibrosis and propose a mechanism of action of the drug using two patient-derived models. The data support the possibility of using this drug as a potentially safe and effective therapy in liver disease patients.

keywords: hepatic stellate cells, extracellular matrix, decellularisation, tissue slices, liver fibrosis

94238159166

HIGH-EFFICIENCY MICROFLUIDICS-BASED MRNA REPROGRAMMING FACILITATES A LARGE COHORT OF PATIENT STUDIES

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The emergence of human induced stem cells (hiPSCs) circumvents the ethical controversy of human embryonic pluripotent stem cells(hPSCs), introducing great feasibility in stem cell research. There are various methods to trigger the cellular reprogramming process, including viruses, plasmids, episomal plasmids, RNAs, proteins, and small molecules. mRNA-based reprogramming has up to 4% efficiency (0.01%~0.1% generally), and iPSCs colonies emerge faster without any genome integration; Moreover, mRNA can be easily produced by any biological laboratory, which increases the flexibility of this strategy. The effective delivery of these pluripotency-related transcription factors is critical for successful reprogramming. Thus, we employ our expertise in microfluidics in mRNA reprogramming, enabling large-scale and high-efficiency production of high-quality hiPSCs. By implementing the reprogramming procedure inside a confined microfluidic chamber (27 mm2-channel, only 1500 fibroblasts required, and 20μL medium consumption daily), we find that in 15 days the yield of hiPSCs has elevated >50 folds compared to the standard wells. Also, the low number of cells required makes reprogramming feasible even when there is limited availability of biological samples. The volume of the system (~5 μL/channel) reduces the cost of reagents >100-fold with a considerable effect on the feasibility of reprogramming a large cohort of patients' cells to study pathology. For example, high-passage fibroblasts from Alzheimer's patients (AD, ~70 years old) have been converted into iPSCs in our microfluidic chips. AD is a neurodegenerative disease that induces progressive dementia in elder people. Unclear pathology of AD hampers our prevention at a young age. However, AD-iPSCs allow us to build the disease models, studying its pathology from the early pluripotent stage with a specific genetic and epigenetic mutation. Disease-specific iPSCs also help to test the efficacy of current drugs and the development of new medicine. In terms of obtaining patient cells, fibroblasts(FB) are from biopsies, which bring invasive damage to the patients, especially to kids, thus peripheral blood monocyte cells(PBMCs) are an ideal alternative cell supply. Blood collection is less invasive, also PBMCs carry fewer gene mutations. Blood outgrowth endothelial cells (BOECs) isolated from PBMCs are an optimal cell source to produce iPSCs. They are an adherent cell population compared to T, B, or other circulating cells in the blood. Besides, BOECs are highly proliferative and less resistant to exogenous mRNA transfections. BOEC-iPSCs start to appear fastly in our microfluidic chips on day 12 when only daily mRNA transfection is performed. Furthermore, instead of verifying the pluripotency of AD-iPSCs, DMD-iPSCs, and BOEC-iPSCs via 2D differentiation, we use the 3D organoids technique to fully characterize their pluripotency and differentiation ability. Our organoids harbor a group of self-renewing FB-iPSCs or BOECs-iPSCs, resembling similar organ physiological conditions in vivo, and are proven capable of all 3 germ layer differentiation. Various types of patient cells have been efficiently reprogrammed into iPSCs in our microfluidic chips by mRNA. It is both time and cost-saving, facilitating the disease pathology study and regenerative medicine research.

keywords: microfluidics, reprogramming, patient cells

PS16
Biomaterials from nature based on extracellular matrices: engineering, repopulation and regenerative potential

62825441319

3D BIOPRINTED SCAFFOLDS BASED ON FUNCTIONALISED BIOPOLYMERS FOR SOFT TISSUE ENGINEERING

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Introduction. In the recent years, three-dimensional (3D) bioprinted hydrogels has been developed and tested for regenerative medicine due to their ability to mimic the anatomical structures [1,2]. Various bioinks that contain polymeric biomaterials, especially natural polymers have been prepared and tested[3] due to their biocompatibility and favorable environment for cell attachment and proliferation[4]. This paper presents the obtaining of 3D bioprinted scaffolds based on combinations between gelatin methacrylate (GelMa), chitosan methacrylate (CsMa), xanthan methacrylate (XMa) or dextran methacrylate (DMa). The scaffolds were obtained by extrusion bioprinting method, were characterized and compared to establish the optimal composition of the bioink and their applicability in soft tissue engineering.

Methodology. The polymers (gelatin, chitosan, xanthan, dextran) were methacrylated according to the protocol described by Camci-Unal [5], adding some changes to the method. Further, GelMa and the other functionalized polymeric (CsMa, XMa and DMa) solutions in PBS were mixed in different concentrations with a photoinitiator (Lap - Lithium phenyl-2,4,6-trimethylbenzoylphosphinate), homogenized, bioprinted and finally freeze-dried for characterization. Bioprinted scaffolds were characterized for their structure, morphology (SEM microscopy), swelling behavior in simulated physiological conditions, in vitro degradability, bioadhesion and in vitro citocompatibility.

Results.The polymers structure and the interactions between their chains were confirmed by FT-IR and NMR spectroscopy and indicate a 3D network with a high stability. Scanning electron microscopy indicated that GelMa reacted with CsMa, XMa and DMa, leading to 3D structures suitable for cells growth and proliferation. The data were supported by the degree of swelling and in vitro degradation tests. All materials were subjected to cytocompatibility and morphological analysis of cell culture. According to the obtained data it was obvious that the scaffolds does not have a cytotoxic effect on the biological elements.

Conclusions. The present paper aimed to obtain scaffolds by 3D printing of new bioinks based on gelatin methacrylate and chitosan methacrylate/xanthan methacrylate/dextran methacrylate. The bioprinted scaffolds have a porosity that support the diffusion of nutrients for cell development; their degradation rate can be controlled by composition and bioprinting conditions. The obtained scaffolds stimulate the cells attachment and proliferation.

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keywords: hydrogels, 3D bioprinting, biopolymers, sorf tissue engineering

73296376419

A FEASIBILITY STUDY OF DEVELOPING CONDUCTIVE, ADHESIVE, REMODELLABLE AND ELASTIC GUMS (CAREGUMS) FOR MENDING BONE FRACTURES

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Bone glues may be the greatest alternative for bonding fractured bone segments compared to metal implants, which require screws, makes the treatment longer, have more adverse-effects for large fracture fragments, and are not suitable for small fracture fragments. The main purpose of this research is to investigate whether a Conductive, Adhesive, Reconfigurable, and Elastomeric material (called CareGum) is applicable as the basis material for bone glue applications. The CareGum material was infused with variable percentages of two different nanoceramic powders to enhance the mechanical behavior, biocompatibility and bioactivity. Several characterization approaches, such as impedance analysis, mechanical, adhesion, self-healing tests, and biocompatibility investigations, were used to evaluate the properties of the CareGum. CareGum samples were immersed in simulated body fluid (SBF), which mimicked the physiological conditions inside a body, to assess the bone bioactivity of the material (ability to stimulate hydroxyapatite (Hap) mineralization). The progression of the CareGum properties after immersion in the SBF was also analyzed. This enabled data collection on the behavior of the material under simulated body physiological conditions. Given the importance of achieving a material that is capable of inducing biomineralization, the optimization of the CareGum formulation and the bioactivity test has been carried out. Overall, the hybrid formulation containing nanoceramic powders can efficiently enhance the mechanical properties of the CareGum more than 100% after immersing in SBF. Moreover, the conductivity of the composite was around 10-3 S/cm, which decreased during the formation of HAp and this can be use to detect the bone formation stage.

keywords: Bone formation monitoring, bone glue, conductive polymer

73296313505

A FIBROUS NATURE OF HYDROGELS PROMOTES DIRECTED MIGRATION OF SCHWANN CELLS

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Introduction
Hydrogel fillings have been successful in enhancing artificial conduits for nerve injuries, but the ideal hydrogel is yet to be found. Its discovery is impeded by an overflow of available products as well as a lack of in vitro experiments that fully analyze the effects of these hydrogels on cells crucial for nerve regeneration.

Methodology
This study investigated various hydrogels advertised for peripheral nerve regeneration. Schwann cells were seeded on the hydrogels and their morphology, viability, proliferation and migration were examined in vitro.

Results
Our results demonstrated that the basement membrane extract hydrogel Cultrex® 3D Culture Matrix® (TrevigenTM) promoted elongated morphology and directed migration in SCs. To elucidate the possible reasons behind this, detailed material characterizations of the hydrogels were conducted and identified highly bundled networks of fibres in Cultrex® 3D Culture Matrix® as well as strain-stiffening. In a next step, we further analyzed two more hydrogels consisting of solely one component of Cultrex® 3D Culture Matrix® respectively namely laminin and collagen and found that the SCs also migrated more directed in the laminin hydrogel but not in the collagen hydrogel. Interestingly, out of the two hydrogels only the laminin hydrogel showed a similiar fibrous surface roughness to Cultrex® 3D Culture Matrix® and also exhibited strain-stiffening. This indicates a correlation between a fibrous nature of hydrogels, strain-stiffening and directed migration in Schwann cells.

Conclusion
These experiments provide a unique systematic assessment of various hydrogels in respect to their material properties and their effect on cells thereby paving the path for the future manufacturing of tailored biomaterials.

keywords: Hydrogels, Nerve regeneration, Tissue Engineering, Biomaterials, Strain-Stiffening, Migration

94238148755

AUGMENTED BONE REGENERATION OF SUPERCRITICAL CARBON DIOXIDE DECELLULARIZED BONE MATRIX SEEDED WITH ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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A novel decellularized bone matrix was established by the supercritical carbon dioxide (SCCO2) extraction technology. The most commonly employed surgical technique is bone grafting, to enhance bone regeneration in orthopaedic surgery. The current study investigated the role of SCCO2 -decellularized bone matrix (scDBM) as bio-scaffold seeded with allogeneic adipose-derived mesenchymal stem cells (ADSC) for bone regeneration. The scanning electron microscopy (SEM) was used to evaluate the bio-scaffold produced by seeding ADSC to scDBM. The bio-scaffold was used to treat the rat segmental femoral defect model in a non-union model to examine the callus formation. Post-surgery after 12 and 24 weeks the osteotomy gap closure and defect area were analyzed using histological analysis. The ability of new bone formation by scDBM was evaluated by the immunohistochemical expression of Ki-67, BMP-2 and osteocalcin. A dose-dependent ADSC was found to adhere definitely to the scDBM bioscaffold as evidenced by SEM. The X-ray and H&E staining revealed callus formation and accelerated bone formation was detected in scDBM seeded with 2x106 and 5x106 ASCs group. The expression of Ki-67, BMP-2, and osteocalcin was increased scDBM seeded with 5x106 ASCs group at 12 weeks after surgery, compared to other experimental groups. To conclude, scDBM is an outstanding bio-scaffold that boosted the attachment and recruitment of mesenchymal stem cells. scDBM seeded with ASCs augmented new bone formation.

keywords: supercritical carbon dioxide (scCO2); decellularized bone matrix (scDBM) scDBM; adipose-derived mesenchymal stem cells (ADSC); bone regeneration

20941818328

BIOCOMPATIBILITY OF DUAL CROSS-LINKED GELATIN-ALGINATE HYDROGELS

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Introduction:Hydrogels are biomaterials that possess appropriate physicochemical and mechanical properties enabling the formation of a three-dimensional, stable structure used in tissue engineering, providing an environment conducive to the survival and proliferation of cells. The cytotoxicity test is performed to assess the overall toxicity of developed materials. Evaluation of the cytotoxicity of the tested materials is quantified by the MTT test. This test uses the fact that the dehydrogenase found in living cells reduces the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoline bromide, known as MTT reagent, to purple formazan. Enzymes from the dehydrogenases group perform the reaction in which the tetrazole ring opens. Thus the cytotoxicity effect is based on the assumption that only living cells reduce MTT to formazan. Biocompatibility tests of the gelatin-alginate hydrogels cross-linked covalently by squaric acid – SQ, dialdehyde starch – DAS, and ionically by calcium chloride – CaCl2 (5%, 2,5%, 1%) treatment were tested because of their significance for regenerative medicine applications. Extracts cytotoxicity was tested on Mouse embryonic fibroblasts - 3T3 and Keratinocytes - KerCT 4048 cell lines.

Methodology:The experiment consisted of 6% gelatin and 2% sodium alginate based hydrogels, DMEM / F12K medium containing 10% FBS, and 1% mixture of antibiotics (identical to the 2D culture medium), and the addition of cross-linkers. Appropriate amounts of polymers and cross-linker were dissolved in the medium, then 30,000 cells / 1.5 ml of final gel were added, and the finished mixtures were poured, onto 6-well plates to form a thin layer. The gels were left for a few minutes to stabilize the shape of the gels and then cross-linked with a CaCl2 solution at room temperature. After 10 min, gels were washed with PBS, and 2 ml of culture medium was added, to each well. The MTT test was performed based on ISO 10993 standard.

Results:The cell viability was reduced after 24h of incubation. An unfavourable effect of a high concentration of Ca2+ ions on cell survival was observed, especially in the case of the KerCT 4048 cell culture. The survival of 3T3 cells grown in an extract of chemically non-cross-linked and cross-linked hydrogel oscillates around 70%. Reducing the concentration of CaCl2 had a positive effect on 3T3 cells. These cells exposed to extracts from the base samples G6_A2_CaCl2 1%, and 2.5% revealed stable and high survivability comparable with control. The addition of chemical cross-linking agents only slightly affected the viability of 3T3 cells. The KerCT 4048 exposed to extracts from G6_A2_SQ1 CaCl2 1% presented the highest viability for this cell line.

Conclusion:Based on the conducted research, gels containing 2% sodium alginate and 6% gelatin presented promising results for future medical applications. Lowering the concentration of CaCl2 solution from 5% to 1% reduced the toxicity of the final material. All significant values in the MTT test remained above the 70% viability established by the ISO 10993 standard.

Therefore, all tested gelatin-alginate hydrogels can be treated as biocompatible.
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keywords: hydrogels, extracts, cytotoxicity, biocompatibility

20941839067

BIOINK BASED ON THE DECM FOR 3D-BIOPRINTING OF BIONIC PANCREAS - FIRST RESULTS OF ANIMAL

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*Purpose: Tissue engineering is an interdisciplinary field involving activities based on two interrelated areas: developing new methods to repair, regenerate and replace damaged tissues and organs. Currently, the most suitable materials for bioprinting of living constructs are biomaterials based on decellularized extracellular matrix (ECM). In this researches, we made two independent hypotheses: (1) that bioinks based on ECM obtained from pancreatic organs should provide structural elements that improve the functionality of pancreatic islets in vitro and (2) the produced bioink should be biocompatible and safe for living organisms, which must be tested on animals.

*Methods: The bioinks used in this study were: 3% alginate, 3% alginate with the addition of gelatin, 3% alginate with the addition of proteins from the extracellular matrix of the pancreas, commercially available bioink with cellulose nanofibrils and our proprietary pancreatic dECM bioink. The islets were subjected to the bioprinting process in each of the above-mentioned bioinks. The petals and islets of the control group were then grown on the inserts immersed in the growth medium for 7 days. After the 7th day of testing, both the petals and the control islets were stained with FDA/Pi and GSIS to demonstrate their viability. Surface and transmission electron microscope was preformed. Bio ink was bioprinted and transplanted into BALB/c mice and observation period was one year.

*Results: There were no statistical difference in GSIS results after bioprinting procedure and control islets after 3Dbioprinting of islets. On the 7-th day post 3Dbioprinitng GSIS was significantly higher in islets bioprinted in our dECM bioink in comparison to control group and other bioinks and was respectively in high glucose: 30 uU/ml vs 5 in control and 4 in other groups.

*Conclusions: The bioink produced by the authors allows for bioprinting of complex tissue systems together with pancreatic cells and islets, while improving their functionality by recreating the natural environment of the pancreas.

keywords: Bioink, extracellular matrix, islets

31412720022

BIOLOGICAL AND MECHANICAL CHARACTERIZATION OF A DECELLULARIZED PORCINE ESOPHAGEAL BIOLOGICAL MATRIX

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Introduction

In pathologies of esophagus the restoration of digestive continuity after the ablation of a portion of the organ is currently ensured by the interposition of a colon segment or by the tubulation of the stomach. The development of a tissue-engineered esophageal substitute is part of the improvement of the surgical treatment of esophageal pathologies. The first objective of this study is to develop a decellularized porcine esophageal matrix able to be cellularized. Then, objectives are to characterize biological and mechanical properties of the decellularized matrix (DM) and the immunogenic potential of mesenchymal stem cells of Wharton jelly (MSC-WJ) using for cellularization.

Methodology

Esophagi were collected from pigs; several steps were required to design a sterile DM: a decontamination process with antibiotics/antimycotics solutions, a decellularization process with a successive perfusion of chemical and enzymatic solutions, a detoxification process, and a sterilization process using peracetic acid (DM-PA).

Biological and mechanical characterizations

Histological analysis

DM (n=6) and DM-PA (n=6) were embedded in paraffin, cross-sectioned and stained with hematoxylin eosin saffron (HES). The efficacy of the decellularization process and the histological structure were then analyzed.

Cytotoxicity analysis

BALB/3 T3 cells were seeded in direct contact with the DM (n=3) and DM-PA (n=3) following ISO 10993-5 standard. Viable cells were counted after neutral red staining.

Mechanical tests

Suturability retention and tensile failure were evaluated with the Shimadzu system (Autograph, AGS-X series) for native esophagus (NE) (n=15), DM (n=30) and DM-PA (=9).

Immunogenicity analysis of MSCs-WJ

Human leucocyte antigens (HLA) of class II (HLA-DR) of MSCs-WJ were immuno-labeled and cells were analyzed by flow cytometry (Canto II Thor). Then, a co-culture analysis with peripheral blood mononuclear cells (PMBCs) was carried out to determine the activation of T cells when in contact with MSCs-WJ.

Results

No remaining cells were observed in HES, and the cohesion of the tissue was preserved after the decellularization and sterilization. The non-cytotoxic character of the DM and DM-PA were confirmed with more than 70% of cells viable when in contact with the DM and DM-PA. For traction tests, there was no significant difference observed for tensile failure between DM (n=30) and DM-PA (n=9), which respectively required a mean force of 33.22N ± 10.90N and 35.12N ±

7.45N. Tensile failure for native esophagus (NE) was obtained for a maximum value of 17.57N ± 6.84N (n=15). For suturability tests, there was no significant difference; DM (n=20) and DM-PA (n=7) had respectively a mean suture retention force of 6.25N ± 1.56N and 5.77N ± 1.03N, for which compared to the NE data (6.89N± 1.77N). Phenotyping of MSCs-WJ demonstrated that only 0.3% of cells expressed HLA-DR. PMBCs expressed a very weakly T cell markers, such as CD69 and CD25, which confirms the immuno-privileged character of these MSCs-WJ for the cellularization of the DM.

Conclusion

We obtained a decellularized and non- cytotoxic biological matrix, able to be recellularized by MSCs-WJ with low immunogenic potential. This matrix preserves the mechanical properties of the native organ, which will promote its tissue and functional integration.

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keywords: decellularized matrix, esophagus, cellularization, MSCs-WJ

52354508919

BIOPRINTING OF BIOACTIVE TISSUE SCAFFOLDS FROM ECOLOGICALLY-DESTRUCTIVE FOULING TUNICATES

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Urochordates are the closest invertebrate relative to humans and commonly referred to as tunicates, a name ascribed to their leathery outer “tunic”. The tunic is the outer covering of the organism which functions as the exoskeleton and is rich in carbohydrates and proteins. Invasive or fouling tunicates pose a great threat to the indigenous marine ecosystem and governments spend several hundred thousand dollars for tunicate management, considering the huge adverse economic impact it has on the shipping and fishing industries. In this work [1], the environmentally destructive colonizing tunicate species of Polyclinum constellatum was successfully identified in the coast of Abu Dhabi and methods of sustainably using it as wound-dressing materials, decellularized extra-cellular matrix (dECM) scaffolds for tissue engineering applications and bioinks for bioprinting of tissue constructs for regenerative medicine are proposed. The intricate three-dimensional nanofibrous cellulosic networks in the tunic remain intact even after the multi-step process of decellularization and lyophilization. The lyophilized dECM tunics possess excellent biocompatibility and remarkable tensile modulus of 3.85 ± 0.93 MPa compared to ~0.1–1 MPa of other hydrogel systems. This work demonstrates the use of lyophilized tunics as wound-dressing materials, having outperformed the commercial dressing materials with a capacity of absorbing 20 times its weight in the dry state. This work also demonstrates the biocompatibility of dECM scaffold and dECM-derived bioink (3D bioprinting with Mouse Embryonic Fibroblasts (MEFs)). Both dECM scaffolds and bioprinted dECM-based tissue constructs show enhanced metabolic activity and cell proliferation over time.
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keywords: Scaffolds, Tunicates, Marine biomaterials, Bioprinting, Tissue engineering

31412769867

CARTILAGE DERIVED EXTRACELLULAR MATRIX INCORPORATED SILK FIBROIN HYBRID SCAFFOLDS FOR ENDOCHONDRAL OSSIFICATION MEDIATED BONE TISSUE REGENERATION

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INTRODUCTION: Hypertrophic chondrocytes mediate the enduring pathway for endochondral bone formation. However, challenges in tissue-engineered bone constructs include functionality under the mechanical environment inside in vivo conditions. We aim to fabricate a mechanically stable Silk fibroin (SF) carrier incorporating decellularized cartilage derived extracellular matrix (CD-ECM) and hypertrophic chondrocytes as a model of endochondral ossification and characterize superior bone-like tissue formation non-destructively in the case of in vitro studies to find the optimal time point when the constructs should be implanted.
METHODS: Human bone marrow stem cells (hBMSC's) were seeded onto CD-ECM/SF or SF constructs and primed for 2-week chondrogenesis following a further 8-week hypertrophy or 4-week hypertrophy + 2-week osteogenesis in differentiation medium. Biochemical assays, SEM/EDX, RT-qPCR, Biomechanical tests followed by non-destructive mCT scanning were used as methods at 4 and 8 weeks to determine hypertrophy mediated ossification.

RESULTS: Calcium deposition biochemically determined increased significantly from 4-8 weeks in both SF and CD-ECM/SF constructs ($p < 0.05$) and retention of sGAG's were observed in CD-ECM/SF constructs. SEM/EDX revealed calcium and phosphate crystal localization by hBMSC's under all conditions. Compressive modulus reached a maximum of 70 KPa in 8-week hypertrophic induction group than 4-week hypertrophy+2-week osteogenesis group ($p<0.05$). mCT scanning at 8 weeks indicated a cloud of denser minerals in groups only induced for 8-week hypertrophic induction. Gene expression by RT-qPCR revealed that hBMSC's expressed hypertrophic markers VEGF, COL10, RUNX2 but absence of early hypertrophic marker ChM1 and presence of later hypertrophic marker TSBS1. Further induction of osteogenesis indicated presence of osteogenic markers ALPL, IBSP, OSX under all conditions.

DISCUSSION & CONCLUSIONS: Our data indicates a new method to prime hBMSC'S into late hypertrophic stage in vitro in mechanically stable constructs for endochondral ossification mediated bone tissue regeneration.

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keywords: Bone Regeneration, Silk Fibroin, ECM, Endochondral ossification

31412736369

CHAMELEON-INSPIRED MULTIFUNCTIONAL PLASMONIC NANOPLATFORMS FOR BIOSENSING APPLICATIONS

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Biomolecule sensing is one of the most fascinating areas in the application of smart biopolymers. One of the most explored areas is the design of soft nanomaterials for glucose monitoring, which is of great significance in the diabetic therapeutic market. Multifunctional biomimetic, biocompatible, and smart materials based on hydrogels with physical properties similar to living tissues are of great significance. In this frame, to develop a platform for sensing the glucose in body fluids, facile and fast sensing, without any external energy and equipment play a vital role.
Here, a platform consisted of two outer layers of plasmonic hydrogel, and one electrospun mat as the inner layer was polymerized using UV light, providing a compact and stable device. Inspired from exceptional chameleon skin features, plasmonic silver nanocubes were embedded in poly(N-isopropylacrylamide)-based hydrogel network to achieve enhanced photothermal-responsive and antibacterial properties. Adequate mechanical and structural properties were provided in the system by the introduction of an electrospun mat into the hydrogel layers, which also creates a compatible environment for homogenous hydrogel coating.
Chemical, morphological and optical tests were performed to investigate the structure of layers and the proposed platform. The synergetic effect of thermo-responsivity and antibacterial properties, as well as sensing features of the nanostructured system, were investigated, revealing that the proposed system is a promising candidate for glucose sensing application.
Acknowledgments: This study was supported by the First TEAM grant number POIR.04.04.00-00-5ED7/18-00, which is conducted within the framework of the First TEAM programme of the Foundation for Polish Science (FNP) and co-financed by the European Union under the European Regional Development Fund.

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keywords: Hydrogel composites, plasmonic nanoparticles, Stimuli-responsive nanomaterials, glucose sensors

73296316806

COLLAGEN-MULTIWALLED CARBON NANOTUBES NANOCOMPOSITE SCAFFOLDS MODIFIED WITH CURCUMIN FOR TISSUE ENGINEERING APPLICATIONS: AN IN-VITRO AND IN-VIVO STUDY

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Introduction: The main aim of tissue engineering is to design biological substitutes in order to enhance and restore damaged tissues or organs, and biomaterials for fabricating the scaffolds play a crucial role. Collagen (COL), the main part of the ECM and a group of structural proteins which contributes to the extracellular scaffolding, was considered for scaffolds preparation. Studies have shown that incorporation of functionalized multi-walled carbon nanotubes (MWCNTs) into various polymeric scaffolds, including COL, can enhance their mechanical, physicochemical, and biological properties.. Several pre-clinical, and clinical studies have shown that curcumin (CUR) has antioxidant, anti-inflammatory, and anti-tumor effects. However, poor bioavailability of CUR owing to decreased serum content, reduced tissue absorption, elevated metabolic rate, and fast removal from the body hampered its practical use. Therefore, incorporation of CUR into scaffolds can enhance its bioavailability at the implantation site. In this study, a novel biomimetic tri-dimensional (3D) scaffolds containing COL (extracted from rat tail), MWCNTs, and CUR were prepared by freeze-drying technique. It was aimed to increase the mechanical properties of the scaffolds by MWCNTs and to promote the anti-inflammatory, bioactivity, and biocompatibility properties of those scaffolds by incorporation of CUR.

Materials and Methods: 0.5 to 1.5% MWCNTs and 5 to 15% CUR were added to the pure COL solutions and freeze-dried. Mechanical characterization of the scaffolds was evaluated by a universal testing machine. Physical and chemical characterization of the scaffolds were also assessed by scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Biological characterization of the scaffolds was also evaluated by in-vitro bioactivity for four weeks, in-vitro biodegradability and the rate of CUR release for 8 weeks, in-vitro biocompatibility using rat synovial-derived mesenchymal stem cells (SMSCs) for seven days, and in-vivo biocompatibility for six weeks.

Results: SEM revealed highly interconnected porosity of the obtained 3D structures with porosity from 75% to 85%.. The addition of up to 1% MWCNTs and 10% CUR has enhanced the mechanical properties of the scaffolds. The presence of MWCNTs and CUR in the COL scaffolds was confirmed by FTIR. The developed COL-MWCNTs composite scaffolds containing 10% CUR revealed excellent in-vitro cytotoxicity and cell attachment using SMSCs. After 8 weeks of examination, the rate of CUR release into the PBS buffer was 20%. Importantly, scaffolds modified with CUR showed reduced inflammatory reactions in the rat model after six weeks of implantation.

Conclusion: CUR incorporated into COL-MWCNTs scaffolds has let to higher mechanical properties, has enhanced the formation of HA crystals on the surface of the scaffold, and has promoted in vitro and in vivo biocompatibility of the scaffolds by making better interaction

between the scaffolds and SMCSs and also by reducing the inflammatory reaction. Overall, the newly developed COL-MWCNTs-CUR 10% freeze-dried scaffolds have demonstrated their high potential for tissue engineering applications.

keywords: Scaffolds; Stem Cells; Collagen; Multi-walled Carbon Nanotubes; Curcumin;

52354533004

CRANIOFACIAL BONE DEFECT REPAIR USING POLYMER SCAFFOLDS AND CELL DERIVED MATRIX

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Introduction
Craniofacial bone defects can be caused from birth defect anomalies and road traffic accidents and affect vital functions such as chewing, swallowing, speaking, and breathing1. Autografts are the current gold standard for treating these defects but suffer from limited tissue available for harvest, long operation times, and donor site morbidity. Tissue-engineering has been known for its potential able to support craniofacial bone regeneration but materials that can surpass autografts advantages have not been identified. Poly(glycerolsebacate)(PGS) is an elastomer generally introduced for soft tissue regeneration which is biocompatible, biodegradable, and tailorable. Porogen leaching with sodium chloride salt is a simple cost-effective technique to fabricate 3D scaffolds. Cell derived extracellular matrix (ECM) after decellularisation has been shown to have a potential of enhancing osteoprogenitor cell attachment, proliferation, differentiation, and mineralisation2. However, decellularised matrix has limited mechanical properties and cannot be shaped to fill complex bone defects. Therefore, we aim to create 3D bone-like tissue for craniofacial repair using a combination of PGS and cell-derived ECM.

Methods
Porous PGS scaffolds were synthesised using a porogen leaching technique with a ratio of 1:3.5 of prepolymer to salt (w/w) and porosity and pore size assessed using pycnometry and scanning electron microscopy (SEM). Y201 (immortalised mesenchymal stem cell (MSC) cell line) attachment and matrix deposition onto the scaffolds were investigated by resazurin reduction assay and Western blot over 21 days. The scaffolds were then decellularised using 20mM ammonium hydroxide with 0.5% Triton X-100 for 24 hours on rocking machine. After being washed with cell culture medium for 72 hours on rocking machine, the scaffold then was soaked in 0.2 mg/ml DNase I solution for 24 hours to help reducing the immunological effect before being washed for another 24 hours. The decellularised and washed scaffolds were then seeded with fresh Y201 cells to elucidate if cell attachment and proliferation are enhanced by the decellularised matrix comparing to cell seeded on fresh scaffolds without any ECM (a control group).

Results
Pycnometer and SEM analysis showed that the ratios used produced a range of pore densities with pore size in the range of 50-400µm. The scaffolds supported cell attachment as assessed by resazurin and the deposition of key bone proteins including collagen, fibronectin, bone sialoprotein 2, and osteopontin as assessed by Western blot. After the decellurisaiotn process and re-seeding cell attachment was higher on scaffold containing ECM but the rate of cell growth was slower compared to the control group.

Conclusion
In summary, salt-leached PGS scaffolds support MSC growth and bone matrix deposition and have potential applications in craniofacial bone regeneration.

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keywords: polymer scaffold, poly(glycerolsebacate), decellularised matrix, craniofacial bone regeneration

41883659229

DECELLULARISATION OF WHOLE HUMAN CONDYLES FOR OSTEOCHONDRAL REPAIR

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Introduction
Osteoarthritis (OA) of articular cartilage is a progressive and debilitating disease, often necessitating a total joint replacement. Between 2005 and 2015 the worldwide prevalence of OA increased by 32.9 % (from 17.9 to 23.7 million cases) [1]. There is therefore a demand for early stage interventions to stave off or delay replacement surgery. A potential treatment is a decellularised osteochondral (OC) graft. Such biological scaffolds should retain their histoarchitecture, biochemical and biomechanical properties, and should not induce negative immune responses when implanted. It is proposed that whole human condyles can be decellularised to produce large, non-immunogenic scaffolds which are then shaped to fit the size and contours of OC lesions.

Methods
Whole medial and lateral femoral condyles (FCs) from were dissected (N=4). The underlying bone was reamed to a total depth of 1 cm. Two medial and two lateral FCs from four separate donors were decellularised and the corresponding lateral and medial FCs retained as in-tissue cellular controls. The decellularisation method applied utilised low concentration sodium dodecyl sulphate (0.1 % w/v), sonication and protease inhibitors. Samples were analysed biochemically (quantitative DNA and glycosaminoglycan (GAG) assays) and histologically to stain for the presence of nuclear material and GAGs (haematoxylin and eosin (H&E), 4′,6-diamidino-2-phenylindole (DAPI) and Safranin O staining).

Results
The total DNA concentration of decellularised bone and cartilage was below the suggested maximum limit of 50 ng.mg-1 dry tissue weight (35.8 ± 12.2 and 4.3 ± 2.3 ng.mg-1 ± SD respectively). GAGs were retained in decellularised cartilage (193.5 ± 37.3 µg.mg-1 (± SD) dry tissue weight; 163.5 ± 42.7 native cartilage). These results were supported by Safranin O staining, which showed GAG retention in the cartilage over the whole sagittal section of the FCs. H&E and DAPI staining showed a reduction in nuclei in decellularised samples; cartilage and bone was largely devoid of visible nuclei. There were occasional nuclei in the calcified tidemark region between the cartilage and bone and the rare occurrence of nuclei in the bone.

Conclusion
FCs were adequately decellularised with excellent GAG retention in the cartilage. As cellular bone allografts are routinely and successfully used in orthopaedic surgery, the presence of the occasional nucleus in the decellularised product should not be of detriment to the immune

compatibility of the graft. It is expected that the removal of cells from an autograft tissue through decellularisation will accelerate healing post implantation, as macrophage mediated removal of necrotic allogeneic cells is not required prior to remodelling of the implanted graft. Future work will seek to explore if the retention of GAGs is accompanied by a retention of mechanical and functional properties, through indentation and friction testing. Decellularised condyles may provide an easily stored, off-the-shelf alternative to viable cellular allografts for the repair of cartilage defects.

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keywords: Decellularisation, Osteochondral, Matrices

73296374439

DEVELOPMENT AND CHARACTERIZATION OF INKS FOR 3D BIOPRINTING IN TENDON TISSUE REGENERATION: NATURAL BIOMATERIALS FOR THE DEVELOPMENT OF BIOMIMETIC SCAFFOLDS

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Tissue engineering has undergone a great revolution in terms of the strategies, technologies and materials it uses (1). In this way, much more complex approximations have been proposed achieving a notorious improvement of the treatment of injuries as frequent as tendinopathies. In this sense, 3D bioprinting could help for the development of scaffolds whose biological or mechanical characteristics resemble those of the native tendon (2).
In this study, different materials of biological origin were analyzed with the aim of obtaining a new ink for 3D printing. The optimal concentrations of each of the materials were studied. The most appropriate protocol was established to guarantee the correct homogenization of the materials and the reproducibility of the ink. Once the final composition of the ink was established, its rheological characterization was carried out. Properties as important as the viscoelasticity and thixotropy of the developed product were analyzed. Next, a printability test was carried out on the 3D printer itself using the developed ink. Finally, tenocytes were incorporated into the ink and 3D bioprinted. The viability and metabolic activity of tenocytes in the scaffold were analyzed.

Hyaluronic acid, alginate, gelatin and fibrinogen were selected as natural materials for the development of the ink. The rotational test allowed establishing that the ink had non-Newtonian behaviour. Its shear thinning behaviour makes it ideal for 3D printing. The initial viscosity at low shear rates was 1020 Pa/s. This ink was not very thixotropic, since after applying and removing the force, the initial viscosity was not fully recovered. The oscillatory test made it possible to establish that the elastic modulus (G') of the ink was greater than the viscous modulus (G'') (elastic solid-like behaviour). As indicated by the rheological studies, the ink could be properly printed. The rheology tests made it possible to establish that the physical characteristics of the inks were dependent on temperature. Through the printability test, this dependency was confirmed and both the ideal temperature and pressure for printing the ink were established (24°C and 120kPa, respectively). After the incorporation of the tenocytes in the ink, the viability and activity of the cells within the scaffolds were analyzed. The viability of the cells at different times was good (no cell death was observed). This result was related to the one obtained for cellular activity in which an increase in activity could be seen over time.

The ink based on hyaluronic acid, alginate, gelatin and fibrinogen presented adequate rheological properties. The ink allowed reproducible designed structures to be printed. The scaffolds obtained were easily manageable and could be manipulated. The mechanical tensile strength was shown to be limited. The good results of cell viability and metabolic activity suggest that the developed scaffolds could have a positive effect on the regeneration of partial tendon injuries. In future studies, the incorporation of nanoparticles with growth factors will be analyzed.

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keywords: Tendon tissue regeneration, 3D bioprinting, biomaterials, Tissue engineering, Bioengineered hydrogels

41883638157

DEVELOPMENT OF A BIOINSPIRED ENGINEERED OVARY TO RESTORE FERTILITY IN CANCER PATIENTS

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Introduction
A major drawback of most chemo- and radiotherapeutic cancer treatments is their toxicity to the ovaries, meaning they may affect fertility and/or endocrine function of female patients. The state-of-the-art method nowadays is the cryopreservation of the ovaries. However, with transplantation of ovarian tissue after the end of cancer treatment there is always the risk to also transfer malignant cells back into the patient which could lead to disease recurrence. A safe alternative to restore fertility in female cancer patients could be to develop a bioengineered ovary using different biomaterials, which would be used as a safe, temporary environment for the patient's follicles. Like the natural ovary, it would contain the patient's follicles, ensure its survival, and support its growth. In this study, we develop 3D scaffolds consisting of different biomaterials to culture cells for several days. We will evaluate cell viability, morphology, and proliferation in response to the different hydrogel formulations.

Methods
Scaffolds for cell culture were fabricated using a combination of the synthetic polymer polyethylene glycol (PEG) and the natural biomaterial collagen type I. Collagen type I was incorporated into the hydrogels to enable cell adhesion within the bioinert PEG hydrogels. Another approach to promote cell adhesion is to incorporate RGD into the hydrogels, which was investigated as well. Different concentrations of collagen were used, and the hydrogels were either supplemented with RGD, or not. In a first approach, hydrogels were seeded with human bone marrow-derived mesenchymal stromal cells (BM-MSCs) at two different concentrations. Cell morphology, proliferation and viability were evaluated over seven days using brightfield imaging, and by quantifying the DNA content of hydrogels directly after fabrication and after one week of culture.

Results
With the help of established protocols to seed PEG hydrogels with BM-MSCs, it was possible to successfully seed cells in PEG-collagen composite hydrogels. Using brightfield imaging it was observed that BM-MSCs start to spread faster in hydrogels containing collagen, compared to those fabricated without. This observation was the same for hydrogels prepared with or without RGD and for both cell seeding densities. Cells proliferated in all hydrogel compositions during seven days of culture, and no difference in proliferation between hydrogels prepared with and without collagen, using the higher cell concentrations, could be detected.

Conclusion
To conclude, a protocol has been established to seed BM-MSCs into PEG-collagen hydrogels with different concentrations of collagen. Future experiments will be carried out incorporating other ECM components into the hydrogels and using fetal membrane cells as more sensitive cells to test for differences in cell proliferation and viability depending on the hydrogel composition.

In addition, matrix deposition of cells will be detected using immunofluorescence staining for fibronectin and subsequent imaging. The morphology of the cells within the hydrogel scaffolds will be evaluated by staining actin and cell nuclei. The stability of hydrogel composition will be evaluated by measuring the collagen content after different number of days in culture.

keywords: hydrogel, ovary, biomaterials

31412719604

DEVELOPMENT OF A BIOMIMETIC IMPLANT WITH STIFFNESS-DEPENDENT IMMUNOMODULATORY FUNCTIONALITY AND NEUROTROPHIC CHARACTERISTICS FOR SPINAL CORD INJURY.

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The injured spinal cord (SC) generates a unique and complex pathophysiology which presents a multifaceted challenge for repair. Broadly, normally supportive astroglia become reactive following injury and form a glial scar. This scar in turn inhibits recovery by releasing an impenetrable inhibitive extracellular matrix (ECM), which prevents axonal regrowth. Tissue engineering approaches aim to overcome these barriers by bridging the cyst with a physical neurotrophic substrate optimised for axonal growth. However, reactive astrocytes rapidly encapsulate these scaffolds, forming a physical barrier to regeneration. Our previous experience in peripheral nerve repair demonstrates that composition and stiffness of scaffolds play an essential role in neural tissue regeneration, directing the behaviour of axons and supporting cells1. Considering the physicochemical properties of SC tissue, we hypothesized that comprehensive optimization of the structure, stiffness and composition of matrix-based biomaterial scaffolds for SC applications could provide potent neurotrophic signalling and intrinsic immunomodulatory properties. Scaffolds with such innate signalling present a simplified alternative to traditional biochemical approaches to addressing the key clinical challenges of astrogliosis, the CNS foreign body response and cross-injury axonal regrowth. To assess the trophic and immunomodulatory properties of native spinal cord materials, neurons and primary human astrocytes were cultured for 7 days on ECM-coated (5 μg/ml) coverslips, or with increasing gradients of biochemical factors and analyzed using immunohistochemistry. To produce biomimetic scaffolds of varying mechanical stiffnesses, soft (3mg/ml), medium (5 mg/ml) stiff (10 mg/ml) hyaluronic acid (Hya) hydrogels were prepared, after which the optimal ECM composition was mixed throughout the hydrogel. The resulting slurry was freeze-dried at -40˚C to form macroporous scaffolds, whose physicochemical properties were subsequently analyzed. Astrocytes and adult dorsal root ganglia (DRGs) were then seeded individually on the HyA scaffolds of varying stiffness and cultured for 1 - 3 weeks. ELISA analysis was performed to examine the release of inflammatory markers IL-6 and IL-10 from the scaffold-resident astrocytes. Immunohistochemistry was performed to examine cell morphology, nuclear:cytoplasmic ratio and axonal extension.

A novel mix of collagen-IV and fibronectin (Coll-IV/FN) was found to optimally enhance neuronal, astrocyte and DRG growth and induce morphological features typical of resting astrocytes. When incorporated into porous hyaluronic acid scaffolds of varying stiffness (0.8-3KPa), Coll-IV/Fn matrix-enhanced scaffolds promoted a range of growth-promoting behaviors in seeded cells in a stiffness-dependent manner. Softer scaffolds with properties similar to the native tissue were shown to mediate astrocyte polarization and significantly increased IL-10 production. Furthermore seeded DRGs exhibited alignment of neurites through the aligned pore structure and significantly increased axonal extension. These results indicate that the interaction of stiffness and the biomaterial surface plays an essential role in mediating repair-critical cellular responses and demonstrates the benefits of a biomimetic approach to the design of scaffolds for SCI repair2.

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keywords: Spinal cord repair, Biomaterial, Immunomodulation, Neural

31412789739

DEVELOPMENT OF A GELMA-PECTIN-BASED HYDROGEL MATERIAL FOR SOFT TISSUE DRESSINGS.

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Introduction:
Breast cancer is the most frequent cancer among women. New therapeutic approaches need to be introduced to develop an effective cancer therapy, which could inhibit, or reverse cancer spread to distant locations. The most prevalent cancer treatment method is resection, during which the tumor with the adjacent tissue (margin) is removed. In such case, patient-tailored hydrogel matrices could be implanted into the resection area, preventing remaining cancer cells from spreading and increasing the tissue's regenerative potential. In this work, pectin from citrus peels, selectively reducing the proliferation of breast cancer cells [1] was introduced into the gelatin methacrylol (GelMA) network in order to obtain base matrix for breast soft tissue dressing.

Methodology:
GelMA hydrogels at concentrations of 5 and 10% (w/v), without or with the addition of 1 and 2% (w/v) pectin from citrus peel were used. For this purpose, GelMA was synthesized, solutions were prepared, and the crosslinking time of the samples was optimized (60 and 90s) using ultraviolet light and calcium ions (for specimens with pectin addition). The samples were subjected to stability testing in the cell culture medium for up to 21 days to determine which material, when used as a dressing/scaffold, would withstand the period of tissue replacement and regeneration. At the same time, DMA testing was performed to determine the mechanical properties of hydrogels and the effect of an additional polymer network- pectin on the material's stiffness. Then, the cross-sectional morphology of the samples with the highest stiffness and stability was analyzed using a scanning electron microscope. The hydrogel solutions were then examined for rheological properties to determine the viscosity of the materials at room temperature. Based on this, three materials crosslinked for the 90s were selected: 5% GelMA; 5% GelMA with 2% pectin; and 10% GelMA and were optimized for 3D printing purposes.

Results:
The specimens prepared from 5 and 10% GelMA and crosslinked for the 90s showed the highest stability. Additionally, it was noted that the longer crosslinking time of the samples allows for a more stable structure that can survive up to 21 days in the cell medium environment, exhibits greater stiffness after fabrication and 24h incubation in fluids. The scaffolds made of 5% GelMA with 2% Pectin and 10% GelMA were found to have the best printout quality with respect to the set parameters.

Conclusions

GelMA-pectin hydrogels were successfully developed in this work. The addition of 2% pectin from citrus peel into GelMA 5% led to stability improvement of the constructs. These materials can be successfully applied for soft tissue dressings, which can be implanted to the operated area after tumor resection.
Further studies on evaluation of the pectin cytotoxicity, and the influence of pectin presence on proliferation and expression of specific genes of cancer cells (studies using tumor cells in 3D cultures) are ongoing.

[1] – F. Salehi, H. Behboudi „Oxidative DNA damage induced by ROS-modulating agents with the ability to target DNA: A comparison of the biological characteristics of citrus pectin and apple pectin"

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keywords: gelatin methacrylol, hydrogels, pectin from citrus peels, tissue engineering, tumor dressings

62825478129

DEVELOPMENT OF AN AUTOMATED SYSTEM FOR EFFECTIVE AND REPEATABLE DYNAMIC URINARY BLADDER DECELLULARIZATION

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Introduction: Urinary bladder regeneration using tissue engineering is one of the major challenges of reconstructive urology. Commonly used urinary bladder reconstruction techniques that utilize gastrointestinal tissues, are associated with many complications. One of the most promising alternative seems to be the use of bladder acellular matrix (BAM). The use of BAM in clinical practice requires the construction of an automated system that will enable bladder decellularization
in an efficient and reproducible manner.

The aim of this study was to develop an automated system that would enable a simple, reproducible and economically viable process for decellularization of the entire urinary bladder and to compare its effectiveness on pig and human urinary bladders.

Methodology: Porcine urinary bladders (n=20) were obtained from a local slaughterhouse and human bladders (n=10) were obtained from cadaveric donors. Firstly, we designed and constructed a homemade whole urinary bladder decellularization system on which a chemical decellularization protocol was developed on a porcine bladder(n=20) using trypsin-EDTA to remove urothelium, hypertonic buffer, Triton X-100 or SDS in hypertonic buffer to remove membranous and cytoplasmic materials. Then, on the basis of experience, a system for automatic decellularization was designed together with Zellwerk GmbH (Germany). Scaffold rinsing was optimised to remove cellular debris and of detergent residues. BAM acellularity was analyzed by histological stainings including HE and Trichrom. BAM structure was evaluated by Scanning Electron Microscopy. The presence of residual DNA was analysed using DAPI staining and molecular analysis. DNA was isolated using DNeasy Blood and Tissue Kit (Qiagen, Germany) according to manufacturer's protocol and quantified using NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, USA). In addition, BAM cytotoxicity against Adipose-Derived Mesenchymal Stem/Stromal Cells (AD-MSCs) in vitro was analyzed using MTT. A content of the BAM components, including collagen, elastin and laminin, were assessed using Collagen Assay Kit (Sigma-Aldrich, USA), Fastin™ Elastin (Biocolor Ltd., UK) and ELISA Kit for Laminin (Cloud-

clone, USA). Additionally, the porcine BAM cytotoxicity, genotoxicity and systemic toxicity were analyzed according ISO-10993 standard in accredited laboratory.

Results: Decellularization was performed using an automated system on porcine and human bladders. According to the results, the automated decellularization system in which decellularization and washing were performed for 3 weeks showed the absence of cell nuclei, and high efficiency of DNA removal, a residual DNA content < 50ng/mg dry tissue weight for both bladders. The viability of AD-MSCs after incubation with BAMs extract obtained by this method was over 90% and 80% for porcine and human BAMs respectively. Additionally, tests in an accredited laboratory have shown that porcine BAM is non-cytotoxic, non-genotoxic and shows no systemic toxicity.

Conclusions: The research carried out allowed the design of an automated system for a simple, reproducible and cost-effective method of decellularizing the entire urinary bladder of both pigs and humans. Due to the wide variation in human bladder structure resulting from individual differences, decellularization of the human bladder is a more difficult process.

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keywords: extracellular matrices, scaffold, urinary bladder

62825417706

DEVELOPMENT OF UDECM AND SACCHACHITIN COMBINED WITH PLATELET-RICH PLASMA (PRP) TO ENHANCE DIABETIC WOUND HEALING

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In this study, we utilized the decellularized extracellular matrix (ECM) under the ultrasonication to obtain the ultrasonic decellularized ECM (UdECM). In SDS-PAGE studies, the results demonstrated that UdECM was mainly composed of type I collagen. The mechanical strength increased with the increase of concentration of UdECM in rheology studies. In addition, the scanning electron microscope (SEM) images showed the highly porous structures, which can absorb the large amount of fluid and cells attached. In diabetic wound healing study, the PRP-loaded UdECM and sacchachitin (SC) hydrogel exhibited the synergistic wound healing on the 14th day. Based on the results, PRP-loaded SC and UdECM showed great potential in future clinical applications.

keywords: Diabetic wound healing, ultrasonic decellularized ECM, sacchachitin , PRP

94238146207

ENGINEERING THE LIVER USING SELF-ASSEMBLED PEPTIDE HYDROGELS

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INTRODUCTION: Liver diseases are becoming a significant public medical burden[1]. To overcome the lack of donor livers and high cost of therapy, this project aims to exploit a long-term culture system that maintains hepatocyte specific functions [2] and cell viability for liver regeneration using self-assembled peptide hydrogels to mimic the normal hepatic extracellular matrix (ECM). Previous work [3] proved that a three-dimensional cell culture environment could keep differentiated hepatocytes compared with in vitro cell culture on a flat surface.

METHODS: All tested self-assembled peptide hydrogels, PeptiGel® Alpha1, Alpha2, Alpha4, Alpha5, Alpha6 and Alpha7 were purchased from Manchester BIOGEL. HepG2 cells (1x106) were suspended in 166µl fresh media then encapsulated within 1ml peptide hydrogel to get a homogeneous cell-gel mixture. 100µl of cell-gel mixture were placed into 24-well inserts, with fresh media added to surround each insert and on top of the sample. The viscoelastic properties of peptide hydrogels and the porcine liver tissue were measured using rheometry. The Cell viability at days 1, 3, 7 and 14 were investigated using LIVE/DEAD® cell viability assay with confocal microscopy. QuantIT Picogreen assay was performed to compare the cell proliferation. To investigate hepatic functions, the cell culture medium was collected every 48 hours to test albumin production and urea secretion by using Human Albumin ELISA Kit (ab179887) and Urea Assay Kit (ab83362). Cytochrome P450 Metabolism and drug response were analysed using qPCR at days 1, 3, 7 and 14. Histology staining was used to observe the morphology of HepG2 cells encapsulated within peptide gel.

RESULTS: The theoretical net charge and mechanical stiffness of peptide hydrogels were investigated. A cell viability assay showed that over 14 days in vitro culture, HepG2 cells proliferated within Alpha5 and Alpha7, would survive within Alpha1, Alpha2, Alpha6. HepG2 cells displayed different viability in pH9 Alpha1 and ph4 Alpha1. During 14 days in vitro culture, ELISA assay revealed a significant increase of Albumin production; HepG2 cells maintained urea secretion and the level increased after day7. qPCR determined increased level of RNA expression for CYP1A2, CYP2C9, CYP3A4 and Albumin but no significant difference of CYP2E1 gene level, from day1 to day14. It was observed that cells produced fibronectin, laminin, collagen I and intercellular albumin according to histology staining.

DISCUSSION AND CONCLUSIONS:
Hepatocytes could maintain cell viability and proliferation when encapsulated within negative-charged peptides rather than within positive-charged peptides. Hepatocytes can maintain the main hepatic functions within Alpha7 gel over 14 days. The results show the potential of 3D peptide hydrogels for drug toxicity prediction and liver regeneration. The investigations of hepatocytes drug metabolism are underway.

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2017.
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keywords: Hepatocyte, 3D in vitro culture, Self-assembling peptide hydrogel

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EX VIVO AND IN VIVO ANALYSIS OF A NOVEL PORCINE AORTIC PATCH FOR VASCULAR RECONSTRUCTION

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(1) Background: The aim of the present study was the biocompatibility analysis of a novel xenogeneic vascular graft material (PAP) based on native collagen won from porcine aorta using the subcutaneous implantation model up to 120 days post implantationem. As a control, an already commercially available collagen-based vessel graft (XenoSure®) based on bovine pericardium was used. Another focus was to analyze the (ultra-) structure and the purification effort. (2) Methods: Established methodologies such as the histological material analysis and the conduct of the subcutaneous implantation model in Wistar rats were applied. Moreover, established methods combining histological, immunohistochemical, and histomorphometrical procedures were applied to analyze the tissue reactions to the vessel graft materials, including the induction of pro- and anti-inflammatory macrophages to test the immune response. (3) Results: The results showed that the PAP implants induced a special cellular infiltration and host tissue integration based on its three different parts based on the different layers of the donor tissue. Thereby, these material parts induced a vascularization pattern that branches to all parts of the graft and altogether a balanced immune tissue reaction in contrast to the control material. (4) Conclusions: PAP implants seemed to be advantageous in many aspects: (i) cellular infiltration and host tissue integration, (ii) vascularization pattern that branches to all parts of the graft, and (iii) balanced immune tissue reaction that can result in less scar tissue and enhanced integrative healing patterns. Moreover, the unique trans-implant vascularization can provide unprecedented anti-infection properties that can avoid material-related bacterial infections.

keywords: vascular reconstruction; vascular grafts; xenografts; porcine aorta; bovine pericardium; macrophages; inflammation

62825405949

FABRICATION OF MYOBLAST-PATCH AND CONDUCT AN ELECTRICAL STIMULATION FOR MUSCLE REGENERATION

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Introduction: Excessive muscle damage does not regenerate on its own, so research is being conducted to regenerate the muscle in tissue engineering. In this study, myoblast-patch that mimics muscle fiber was generated and characterized in vitro test.

Method: To generate myoblast-patch, scaffolds were fabricated with biocompatible materials using electrospinning. Two groups of scaffolds were prepared as follows: aligned- and random-fiber-based scaffold. Myoblasts were seeded and cultured on the fabricated scaffold for analysis. And then, Experiments were conducted to observe the cell proliferation, differentiation capacity, and response to electrical stimulation.

Result: As a result, the cell proliferation and differentiation ability were more increased in the aligned Myoblast-patch. Also, the intensity of calcium ion imaging to electrical stimulation was higher in the aligned myoblast patches. Therefore, it is shown that the aligned myoblast patch is more suitable for muscle regeneration.

keywords: myoblast-patch, muscle regeneration, electrical stimulation

52354578579

FABRICATION OF RENEWABLE AND ACTIVE CO2-DERIVED BIOCOMPOSITES BY GREEN AND SUSTAINABLE WATER-BASED PROCESS

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Biocomposites made of poly(propylene carbonate) (PPC), a carbon dioxide (CO2) derived polymer, and a renewable polysaccharide are promising materials for several applications such as biomaterials and packaging. However, most of PPC-based biocomposites exhibit low mechanical and optical properties due to the inherent incompatibility between PPC and most other polymers. In this work, a novel biocomposite composed of PPC and chitosan was developed using a facile and sustainable water-based production process. This innovative synthetic route enables the formation of chitosan micron/submicron particles dispersed within the PPC matrix. This, in turn, leads to improved mechanical performance and thermal stability of these renewable and low carbon footprint materials. Furthermore, vitamin C is incorporated and its sustained release from the biocomposites results in prolonged and effective antioxidant properties. These CO2-based biocomposites are biodegradable in seawater forming non-toxic products. The developed PPC/chitosan biocomposite represents intriguing candidates for the sustainable development of biomaterials

keywords: poly(propylene carbonate), chitosan, biocomposites, active materials, water-base process

31412721205

GENERATION OF DECELLULARIZED SCLEROCORNEAL LIMBI FOR USE IN TISSUE ENGINEERING PROTOCOLS

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Introduction: Damage of the sclerocorneal limbus may lead to limbal stem cell deficiency (LSCD), loss of corneal transparency, and blindness. Keratoplasty is the standard treatment for corneal transplantation disorders, but it is contraindicated in cases with LSCD or limbal damage1, which are very difficult to manage. Generation of a biological substitute of the sclerocorneal limbus could contribute to treat these patients. However, the complex three-dimensional structure of the sclerocorneal limbus makes it difficult to generate fully functional artificial tissues2. In the present work, we describe a bioengineered sclerocorneal limbus substitute generated by decellularization techniques.

Methodology: Four different decellularization protocols were applied to the porcine sclerocorneal limbus. These protocols were based on the use, alone or in combination, of osmotic agents (distilled water and NaCl), detergents (SDS and SD) and enzymes (nucleases) in different proportions to remove native cellular components. The efficiency of the decellularization procedure was assessed by DAPI staining and DNA quantification. Morphology, structure, and ECM composition were assessed histologically. In addition, transparency of decellularized limbus were evaluated.

Results: All protocols achieved effective decellularization, with HE and DAPI analyses showing no remaining cell nuclei in any protocol. However, some differences were found for the DNA quantification analysis, with protocols based on 0.1% SDS showing the best results. Analysis of corneal transparency revealed that all decellularized tissues were able to transmit the incoming light, although the most adequate results were found in the 0.1% SDS group. Interestingly, the analysis of collagen fibers showed that the 0.1% SDS was able to more adequately preserve the amount and orientation of the stromal collagen fibers as compared to the other experimental groups.

Conclusions: Our results demonstrate that decellularized sclerocorneal limbus xenografts can be efficiently generated in the laboratory. Application of specific decellularization protocols based on 0.1% SDS showed the most optimal results in terms of remaining nuclear components, transparency and ECM composition, being a promising alternative for LSCD treatment.

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keywords: Sclerocorneal limbus, Limbal stem cell deficiency, Decellularization, Xenograft.

52354506355

HIERARCHICALLY TARGETABLE POLYSACCHARIDE-COATED SOLID LIPID NANOPARTICLES AS AN ORAL CHEMO/THERMOTHERAPY DELIVERY SYSTEM FOR LOCAL TREATMENT OF COLON CANCER

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Although oral formulations of anticancer chemotherapies are clinically available, the therapeutic action relies on drug absorption, being inevitably accompanied with systemic side effects. It is thus desirable to develop oral therapy systems for the local treatment of colon cancers featured with highly selective delivery and minimized systemic drug absorption. The present study demonstrates the effective accumulation and cell uptake of the doxorubicin and superparamagnetic iron oxide nanoparticles-loaded solid lipid nanoparticles (SLNs) delivery system for chemo/magnetothermal combination therapy at tumors by hierarchical targeting of folate (FA) and dextran coated on SLN surfaces in a sequential layer-by-layer manner. Both the in vitro and in vivo characterizations strongly confirmed that the dextran shells on SLN surfaces not only retarded the cellular transport of the FA-coated SLNs by the proton-coupled FA transporter in small intestine, but also enhanced the particle residence in colon by specific association with dextranase. The evaluation of the in vivo antitumor efficacy of this SLN therapy system by oral administration showed the effective inhibition of colon tumors.1 The SLNs were prepared by emulsion method and sequentially coated with folate-modified D-a-tocopheryl polyethylene glycol succinate and octadecanol-conjugated dextran by hydrophobic interaction. The enzymatic degradability of dextran was demonstrated by the incubation with dextranase, a bacteria-produced glucanohydrolase present exclusively in colon. The cellular uptake examinations were performed by FA receptor (FAR) overexpressing CT26 colon cancer cells. An orthotropic CT26 colon tumor model was established with BALB/c mice. For in vivo distribution in gastrointestinal tract (GI tract), SLNs were labeled with NIR probe to facilitate IVIS fluorescence detection (Xenogen). For in vivo tumor inhibition study, mice were treated with drugs by gavage at a daily DOX dosage of 12 mg/kg for total three doses. To generate hyperthermia effect, the high-frequency magnetic field (HFMF) treatment was performed for 7 minutes. The dextran/FA-modified SLNs (DFSLNs) exhibited the average particle sizes of 132.1 nm and the DOX loading content of 9.27 wt%. The incubation of DFSLNs with dextranase led to the reduction of sizes to 97 nm, similar to the sizes of FA-modified SLNs (FSLNs). The in vitro targeting efficiency of the FA-decorated SLNs to FAR overexpressing CT26 cells substantially enhanced compared to SLNs and DFSLNs. The reduction of cellular uptake of DFSLNs was caused by the shielding effect with dextran that impaired the FA-mediated interactions between the NPs and the cells. As a consequence, after the removal of dextran coating with the enzymatic action of dextranase, the DFSLNs showed comparable uptake to FSLNs. The in vivo distribution of DFSLNs in GI tract shows reduced retention in intestine than that of FSLNs due to the FA residues sequestered by dextran coating. Moreover, DFSLNs promoted colon tumor region accumulation for about 2.8-folds compared to FSLNs in part by evading the undesired biorecognition. In particular, the effective tumor growth inhibition of the orthotopic colon tumor for the tumor-bearing mice receiving the DFSLNs and DFSLNs/HFMF treatments, respectively, clearly demonstrated the prominent therapeutic efficacy of DFSLNs for local treatment by oral administration.

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keywords: Colon cancer; Targeting; Combination therapy; Solid lipid nanoparticles; Oral formulations

73296319089

HYALURONIC ACID/LACTOSE-MODIFIED CHITOSAN ELECTROSPUN WOUND DRESSINGS – CROSSLINKING AND STABILITY CRITICALITIES

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Introduction: Electrospun wound dressings are emerging as promising candidates in the field of wound care since they present a similar architecture to natural extracellular matrix (ECM). Their composition can include both synthetic and natural polymers, the latter enabling a biological other than structural mimicking of ECM. Due to the high surface-to-volume ratio, electrospun matrices can absorb a large amount of wound exudate and favor gaseous exchanges, with an anti-scarring and hemostatic potential. Notwithstanding the considerable benefits of using natural polymers (proteins and polysaccharides), their electrospinnability is impaired by their basically high viscosity, surface tension, or conductivity. For this reason, the combination with synthetic polymers beyond the addition of surfactants has been proven to be an efficient strategy to improve the electrospinning process. Furthermore, the presence of water-soluble polysaccharides affects matrix stability in aqueous environment, thus requiring additional crosslinking steps to stabilize the final structure and allow wound protection.

Methodology: Hyaluronic acid/lactose-modified chitosan electrospun matrices were produced by using polyethylene oxide as synthetic support and Tween®20 as surfactant. Polycaprolactone matrices (pristine, air plasma-activated, or polysaccharide-coated), polysaccharide-based freeze-dried membranes, and Chitoderm® were used as comparison. Considering the polysaccharide-based membranes instability in aqueous environment, several crosslinking strategies were pursued ranging from traditional chemical (glutaraldehyde, genipin, EDC/NHS) and physical (heat) methods to innovative chemical crosslinkers in the field of electrospinning, namely carbonyldiimidazole (CDI) and methacrylic anhydride (MA). The ability of CDI- and MA-crosslinked mats to absorb exudates as well as their degradation behavior were tested both in water and saline solution. In addition, the water vapor transmission ability was evaluated.

Results: Membranes with thin, uniform, and defect-free nanofibers, were obtained. Due to their complete instability in water, different crosslinking methods already reported in literature were attempted, with unsatisfying results. Indeed, glutaraldehyde, genipin, EDC-NHS, or thermal treatment were unable to stabilize the final structure, leading to the loss of the fibrous morphology and to an immediate dissolution in water. For this reason, two novel crosslinkers, namely CDI and MA, were tested, obtaining better results than the traditional methods. Indeed, MA allowed to maintain a proper nanofibrous structure after crosslinking but did not ensure long-term stability in aqueous environment. On the other hand, CDI crosslinking was responsible for a partial loss of the fibrous structure, although guaranteeing its stabilization. This morphological alteration did not affect matrix fluid retention, being three times higher than non-electrospun products and the commercial product Chitoderm®. Likewise, electrospun products revealed an optimal water vapor transmission ability, being halfway between the total evaporation and the absence of transmission.

Conclusions: Polysaccharidic electrospun wound dressings with suitable properties in terms of ECM mimicking, exudate absorption, and gas permeation were produced, by exploiting several crosslinking strategies with the aims of stabilizing the final structure and obtaining an

optimal compromise between nanofibrous architecture maintenance and aqueous stability. The conformability to the wound and the mechanical properties of the synthetized dressings could be enhanced in a biphasic system where the polysaccharidic matrix is linked to a synthetic counterpart, able to provide mechanical strength.

keywords: Polysaccharides; Electrospinning; Wound dressings; Crosslinking; Carbonyldiimidazol

73296322884

HYDROGEL MATERIALS BASED ON CHITOSAN CROSSLINKED WITH FUNCTIONALIZED POLYSACCHARIDES: IN VITRO CYTOCOMPATIBILITY AND CARTILAGE REGENERATION POTENTIAL

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Hydrogel materials are widely used in medicine and tissue engineering due to their similarity to the natural tissues of the human body. They are well known for their high biocompatibility and swelling - the ability to absorb large amounts of water. These properties determine the possibility of the use of hydrogels in the regeneration of cartilage tissue. In order to enhance regeneration potential of hydrogels, they can be modified with different biologically active compounds. Furthermore, when obtaining hydrogel materials for this purpose, it is extremely important to use an appropriate cross-linking agent that will enable the creation of a durable and stable three-dimensional polymer structure. For biopolymers containing amino groups, e.g. chitosan, such a function can be performed by polysaccharides after appropriate chemical modification. Due to the oxidation of polysaccharides, it is possible to obtain dialdehyde polysaccharides (DAP), which in their structure will have reactive aldehyde groups. These functional groups readily react with amino groups by Schiff base cross-linking, which allows to use the DAPs as unique biopolymer cross-linkers.

The subject of research in this study are hydrogel materials based on chitosan cross-linked with functionalized polysaccharides such as chitosan, dextran, furcellaran, gellan gum and xantan gum. Additionally, functional ingredients such as natural polyphenol - rosmarinic acid and bioactive glass doped with strontium were introduced into the structure of the materials.

In vitro tests were carried out on Hs680 fibroblasts and normal human chondrocytes. The proliferation of cells and cytotoxicty of hydrogel materials were monitored. Moreover the ELISA test allowed to assess the concentration of the components of the extracellular matrix in cell culture – collagen type II and aggrecan.

The obtained results show that by appropriate selection of components, it is possible to obtain stable and cytocompatible chitosan-based hydrogel materials supporting cartilage tissue regeneration.

Acknowledgements
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keywords: hydrogel, chitosan, in vitro, cartilage

41883625566

HYDROGELS FOR 3D EXTRUSION PRINTING OF GRADIENT SCAFFOLDS

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Introduction: The advent of 3D printing technology has opened up a new avenue in the field of tissue engineering [1]. The cell-friendly and printable hydrogels are ideal materials for the construction of 3D biomimetic scaffolds for 3D cell cultures. However, the development of bio inks satisfying features of printability, water-retention capacity, biocompatibility, and suitable mechanical properties (i.e. stiffness) and degradability is one of the main challenges that still persist. Therefore, the current study focuses on developing a new bioink formulations based on natural-derived materials (e.g. alginate, gelatin, and hydroxyethyl cellulose) with good printability and tunable stiffness. We aim at obtaining printed hydrogel scaffolds with stiffness gradient.

Methodology:
Hydrogel materials composed of alginate, gelatin and hydro-ethyl cellulose and their functionalized versions (e.g. methacrylate functionalization) were prepared at different compositions. Their rheological properties, printability and stiffness of crosslinked gels were analyzed. The growth and proliferation of cell encapsulated in the scaffold were tested using live dead and alamar blue assay.

Results:
The results generated proved the printability of the proposed formulations with good shape fidelity. Based on rheological analysis, the scaffold showed different stiffness depending on the material composition. Printed hydrogels containing fibroblasts (NIH3T3) demonstrated high cell viability and proliferation in time. Scaffolds with stiffness gradients were successfully obtained.
Conclusion: Hydrogel scaffolds with stiffness gradients form biocompatible environment for 3D cell cultures. In future we will work on the scaffolds with higher complexity, i.e. including biochemical and topological gradients. We envision that this work will be useful for tissue engineering of native gradients structures, e.g. interfacial tissues.

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Reference:
[1]	S. Agarwal, et al., Current Developments in 3D Bioprinting for Tissue and Organ Regeneration–A Review, Front. Mech. Eng. (2020) 90.

keywords: Bio ink, alginate, gelatin, hydroxyethyl cellulose, 3D bioprinting, interface tissue

52354507209

INFLUENCE OF THE PARALLEL HOLES IN THE BONE REGENERATIVE MATERIAL FOR VERTICAL BONE AUGMENTATION

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Introduction: Even though conventionally prepared octacalcium phosphate and collagen composite (OCPcol) has exhibited excellent bone regeneration with angiogenesis (1, 2) and has already been commercialized for treating bone defects (3), reproducible appositional bone formation with OCPcol has never been achieved. Recently, it was developed double concentration of OCP/Col with applying liquid nitrogen during the preparation (OCPcol L2.0). And it demonstrated reproducible appositional bone formation in preclinical study (4). Because it was reported that bone formation in the implant would be enhanced by orderly arranged unidirectional macropores, the present study investigated whether appositional bone formation could be enhanced by different types of unidirectional macropores in OCPcol L2.0.
Methods: The prepared OCPcol L2.0 (diameter 9 mm, thickness 2 mm) were given parallel holes in the axial direction for adjusting cell invasion from outside of the disks, and divided four groups. They were OCPcol L2.0 disks with through hole (open hole: Open), blind holes from top to bottom (Top), blind holes from bottom to top (Bottom), and untreated (Unt). These disks were implanted into subperiosteal pockets in rodent calvaria (n = 5 x 4 groups). Radiomorphometric analysis was conducted at 4 and 12 weeks after implantation, and histological analysis was conducted at 12 weeks after implantation.

Results: In radiomorphometric analysis, the Open group indicated a greater degree of deformation than the other three groups, whereas all groups tended to retain their height and shape. In histomorphometric analysis, appositional bone formation was dominantly initiated from the surface of original bone towards inside of the implants, and the Open group demonstrated the lowest appositional bone formation compared with other three groups.
Conclusions: These results suggest that the addition of through holes in OCPcol L2.0 might be a subordinate factor for appositional bone formation compared with the increase of concentration of OCPcol or application of liquid nitrogen during the preparation.

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keywords: Animal models; Biomaterials; Bone augmentation, Collagen, Octacalcium phosphate

73296324205

NANOFIBRILLATED CELLULOSE-BASED BIOINKS FOR BIOPRINTING AND 3D CELL CULTURE

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The use of xeno-free (animal origin-free) hydrogels to support the growth of cells significantly increased in the recent years. The advantage of these materials in comparison with animal derivates is the reproducibility of structural, mechanical and biological properties and, additionally, overcoming of potential immunological complications and disease transmission in vivo. Many novel commercially available bioinks intended for extrusion bioprinting contain nanofibrillated cellulose (NFC) derived from plants. We performed the comparative testing of several NFC-based hydrogels for their printability and biocompatibily: GrowDexT and GrowInk T (UPM Biomedical) and Laminink+ (CellInk), where the last one is composed of NFC with addition of sodium alginate and laminin. To assess the biocompatibility of tested bioinks, the survival and behavior of immortalized human adipose derived stem cells (hASCs, Evercyte) encapsulated in the bioinks was observed over 14 days using laser confocal microscopy (LSM 700, Carl Zeiss) and metabolic activity testing (PrestoBlue, Invitrogen). The main tuning parameter was the concentration of the bioinks, as it affects the printability of the ink and the viability of the cells inversely. Therefore, not only the printing parameters – pressure, movement speed, preflow delay, nozzle size for extrusion bioprinting (BioX, CellINK) but also the right mixing ratio of bioink and cell suspension were established to obtain stable structures with favorable conditions for proliferation and migration of cells. Performed experiments showed that optimization of the bioprinting parameters and material formulation for each hydrogel are essential for obtaining durable structures and long-term survival of encapsulated cells. From the tested bioinks, GrowDex-T with a concentration of 0.8% w/v, stood out as the best candidate for bioprinting of hASC, as we observed better proliferation and change of cell morphology from round to the spindle shape. An additional advantage of this material is its transparency and non-fluorescence, that facilitates microscopy of 3-dimensional cell loaded structures.

keywords: nanofibrillated cellulose, xeno-free, bioprinting, biocompatibility, 3D cell culture

41883667848

NEW BIOREACTORS FOR RECELLULARIZATION OF PORCINE LIVER SCAFFOLD PIECES

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Introduction
In the last two decades research has been focused on the development of biologically-derived matrices for reconstructive medicine[1]. Decellularized tissues from a xenogeneic donor represent a growth-friendly environment for cells of different species, including human, lacking most of the immunogenic components[2]. Decellularized porcine liver resembles the human counterpart and could be considered for reconstruction of human liver tissue in vitro. Thus there is a high need for bioreactor development where the effective cell repopulation can be achieved using a good quality scaffold.

Methodology
A bioreactor system was designed and constructed for perfusion cell culture in pig liver scaffold pieces with approx. volume 3 cm3. Two types of custom-made chambers with inserts holding four scaffolds individually fed with medium were developed. The circulation of oxygenated medium from a special bottle was enabled by peristaltic pumps. Porcine livers were decellularized with consecutive cycles of 1% Triton X-100 and 1% sodium dodecyl sulphate (SDS) solutions[3]. Then the scaffold was cut and tested in the bioreactor for human cell adhesion and proliferation. HepG2 cells were injected in the scaffold matrix at seeding density of 10^6/cm3, and then cultivated in the bioreactor for 1, 3 and 7 days. Fixed scaffold sections were stained with hematoxylin-eosin to monitor the efficiency of the cell repopulation.

Results
Completely decellularized porcine liver scaffolds with a well preserved matrix showed presence of HepG2 cells in all tested recellularization experiments. This includes both bioreactor chambers as well as all three time points. After one day of incubation, the cells were visible only at the injection site and in adjacent vascular ECM structures. After 3 and 7 days of culture the cells proliferated and started to migrate into the area surrounding the injection site. Some cells were also detected on the surface of the scaffold; these cells were probably released from scaffold into medium during the early perfusion and adhered later. Living cells detected inside the matrix were also indicative of the removal of any residual SDS content after the decellularization. It is known that even very low SDS concentrations can hamper the repopulation efforts, thus washing out the detergent residues is important.

Conclusions
We proved that both bioreactors can support cell adhesion and proliferation in perfusion 3D culture, thus they can be used for the repopulation of small decellularized liver pieces. Moreover, our models can be adapted to different types of tissues to verify the initial potential of the matrix to support cell growth. The ability to repopulate these small decellularized pieces is the first step to proceed firstly with lobules, in the case of decellularized liver scaffold, and then with the entire organ.

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keywords: Bioreactors, decellularized scaffold, scaffold repopulation, decellularized liver tissue

62825437684

OPTIMISING THE FABRICATION, SURFACE TREATMENT AND MECHANICAL STIMULATION TO IMPROVE THE CELL PROLIFERATION AND COLLAGEN PRODUCTION FROM PRIMARY DERMAL FIBROBLASTS IN VITRO

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Natural scaffolds derived from living tissue have been used in clinical applications. They provide similar architecture and composition to the target tissue and can be remodelled as desired. However, they still have some drawbacks as there is a risk of disease transmission and may require additional surgery. Therefore, extracellular matrix (ECM) based biohybrid scaffolds have been developed using synthetic scaffolds as templates. Mechanical stimulation is proved to be helpful in cell proliferation and collagen production. However, there is limited information regarding the most appropriate technique and mechanical regime for generating biohybrid scaffolds. This project focuses on optimising the PGSM scaffolds and mechanical regime to induce cell proliferation and collagen content.

80% Polyglycerol(sebacate)-methacrylate (PGSM) polymer is used for making ECM-based scaffolds as it is biocompatible and biodegradable. 1x105 cells of dermal fibroblasts were seeded on PGSM scaffolds. Sugar-leached and emulsion templating fabrication techniques were compared. Scaffold surfaces were modified by exposure to either Ar or O2 plasma. After three weeks of cell culture, the scaffolds were analysed using resazurin and picrosirius red for cell proliferation and total collagen production, and the impact of different fabrication techniques and surface modifications compared. To investigate the effect of mechanical stimulation, the cell-seeded scaffolds were cultured in an Ebers TC-3 bioreactor. The regimes used either short(10 s stimulation, 30 s rest) or extended (2 h stimulation, 6 hours rest)stimulation and resting periods in combination with 5 or 10% tensile strain. All the regimens used in the experiments had the same total stimulation time, with strain applied at 1 Hz. After two weeks of stimulation, the scaffolds were analysed with resazurin, picogreen, and picrosirius red for cell proliferation and collagen content. 3D static controls had the same number of cells on scaffolds that were not exposed to strain.

80% PGSM scaffolds fabricated by emulsion templating induced higher cell proliferation and collagen production than sugar-leached scaffolds. However, there was no significant difference between surface treatments when compared with the same fabrication technique. Therefore emulsion templating and O2 plasma treatment was used to generate the scaffolds for the remainder of the study Mechanical stimulation results show that with longer active and resting periods, in combination with 10% tensile strain, the cells generated more collagen. However, this regime showed no significant impact on cell proliferation when compared to unstimulated cells. However, shorter stimulation and rest periods, together with 5% tensile strength resulted in significantly greater cell proliferation, but no significant difference in terms of total collagen when compared to results from unstimulated scaffolds.

Mechanical stimulation has been shown to influence the ECM secretion from cells in vitro. To be able to obtain the highest amount of collagen from cells in vitro, the appropriate scaffold and mechanical regime are necessary. It is suggested that one regime can only promote one aspect.

A combination of two regimes will be tested as the next step to enhance both cell proliferation and collagen production.

keywords: mechanical stimulation, plasma treatment, 3D cell culture, polymeric biomaterials, ECM-based scaffold

62825439044

PERSONALIZED BONE MATRIX FOR HUMAN BONE DEFECT REPAIR USING 3D CAD/CAM CARVING AND SUPERCRITICAL CARBON DIOXIDE EXTRACTION TECHNOLOGY

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Supercritical CO2 (SCCO2) technology was used to eliminate the cells, fat and non-collagenous materials while preserving a native collagen scaffold as a biomedical device for bone defects. We used porcine matrix bones with 3D CAD/CAM carving to reconstruct a personalized, wide range and complex-shaped bone. In oral cancer patients upto 30–50% of need mandibulectomy and autologous fibula reconstruction. Autograft is the gold standard preference due to its histocompatibility; but, it needs extra surgery. Allograft and xenograft are substitutes but are vulnerable to immune response. At present, no personalized bone xenografts are accessible in the market for large fascial bone defects. In addition, a large-sized complex shape bone graft is not possible to be manufactured directly from the raw material. We forestall that patients can reestablish their native facial appearance after reconstruction surgery. We effectively established 3D CAD/CAM carved bone matrices, followed by SCCO2 decellularization of those large-sized bones. A lock-and-key puzzle design was employed to fulfil a wide range of large and complex-shaped maxillofacial defects. To conclude, the 3D CAD/CAMcarved bone matrices with lock and key puzzle Lego design were completely decellularized by SCCO2 extraction technology with intact natural collagen scaffold. In addition, the processed bone matrices were tested to show excellent cytocompatibility and mechanical stiffness. Thus, we can overcome the limitation of large size and complex shapes of xenograft availability. In addition, the 3D CAD/CAM carving process can provide personalized tailor-designed decellularized bone grafts for the native appearance for maxillofacial reconstruction surgery for oral cancer patients and trauma patients.

keywords: maxillofacial reconstruction; supercritical CO2; collagen bone matrix; 3D carved bone; cytocompatibility

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POLYVINYL ALCOHOL/GELATIN ELECTROSPUN FIBERS LOADED METHYLPREDNISOLONE VIA HRP-MEDIATED CROSS-LINKING IN SPINAL CORD REPAIR

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We proposed a new formation for fibrous mat fabrication from derivatives of polyvinyl alcohol (PVA) and gelatin (Gela) through horseradish peroxidase mediated crosslinking which provides sustain release of methylprednisolone (MP) as a therapeutic approach for SCI regeneration. The polymeric solution of PVA and Gela conjugated with phenolic moieties (PVAPh & GelaPh respectively) containing HRP and MP flowed using a syringe pump in an electrospinning system. The PVAPh/GelaPh fibrous mats were put in air flow saturated with H_2O_2 which acts as an electron donor and covalently proceed crosslinking of Ph groups in fibrous mats. Implanted scaffolds were then transplanted into the compression SCI rats' model. A total of 40 rats were injured at the T10-11 level, and regarding transplantation materials. After a specific interval, the animals underwent a study for remyelination and apoptosis using TUNEL and Luxol Fast Blue staining as well as behavioral test. The covalently crosslinked PVAPh/GelaPh fibrous mats were in uniform size distribution without any bead formation and maintained their filament structure. The mechanical properties, hydrophobicity and degradation rate of PVAPh/GelaPh fibrous mat are significantly promoted compared with PVAPh samples and could provide the desired structure for sustain release of MP. The cells adhered, proliferated on this composite which indicates cytocompatibility of resultant PVAPh/GelaPh fibrous mats. Our result showed a significant decrease in apoptotic neurons and a remarkable increase in remyelination in SCI+PVA/GELA+MP group compared to the SCI group. Therefore, the results confirmed the potential of PVA/Gela+MP nanocomposites as a scaffold for SCI through locomotor hind limb function amelioration, neuroinflammation attenuation, improvement of functional recovery and limiting the secondary damages.

keywords: Poly Vinyl Alcohol/ Gelatin conjugated phenolic moieties; fibrous mat; Sustained release of methylprednisolone; Spinal Cord Injury

62825421786

REGENERATIVE POTENTIAL OF BMSCS GROWN ON HIPSC-ENGINEERED EXTRACELLULAR MATRIX

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Introduction
Mesenchymal stromal cells (MSCs) have the potential to respond to injury and aid in repair and regeneration of damaged tissues. Bone marrow and adipose tissue are the major sources of MSCs for clinical applications. Previous studies in animal models suggested that the regenerative activity of aged stem cells/MSCs can be enhanced by exposure to biomimetic microenvironments. The aim of our project was to investigate whether extracellular matrix (ECM) engineered from human induced pluripotent stem cells (hiPSCs) can enhance the regeneration potential of human bone marrow mesenchymal stromal cells (hBMSCs).

Methods
ECM was engineered from hiPSC-derived mesenchymal-like progenitors (hiPSC-MPs). ECM structure and composition were characterized before and after decellularization using immunofluorescence and biochemical assays. hBMSCs were cultured on the engineered ECM, and differentiated into osteogenic, chondrogenic and adipogenic lineages. Growth and differentiation responses were compared to tissue culture plastic controls.

Results and Discussion
Decellularized ECMs contained collagens type I and IV, fibronectin, laminin and < 5% residual DNA, suggesting efficient cell elimination. Cultivation hBMSCs on the ECM in osteogenic medium significantly increased hBMSC growth and markers of osteogenesis, including collagen deposition, alkaline phosphatase activity, osteogenic gene expression levels and matrix mineralization compared to plastic controls. hBMSCs cultivated in chondrogenic micromass culture significantly increased growth and markers of chondrogenesis, including glycosaminoglycans and collagen type II deposition, and chondrogenic gene expression. In contrast, adipogenic differentiation of hBMSCs on ECM resulted in significantly increased hBMSC growth on the ECM, but significantly reduced markers of adipogenic differentiation. Together, our studies suggest that BMSCs regenerative activity for osteogenesis and chondrogenesis could be enhanced, whereas adipogenic activity was diminished by the culture on hiPSC-engineered ECM. This counteracted the natural shift in hBMSC differentiation from osteogenesis to adipogenesis during aging. The contribution of specific matrix components and underlying mechanisms need to be further elucidated.

Conclusion
Our studies suggest that osteo- and chondrogenic regenerative activity can be enhanced by hBMSC culture on hiPSC-engineered ECM. Importantly, hiPSCs represent a scalable, clinically-relevant source for tissue engineering strategies employing engineered ECM materials.

keywords: Trilineage differentiation, human bone marrow stromal cells, extracellular matrix, hiPSCs

94238142077

SUPERCRITICAL EXTRACTION OF ECM COMPONENTS FOR BIOINK DEVELOPMENT

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Introduction
Extracellular matrix (ECM)-based bioinks have been steadily gaining interest in the field of bioprinting to develop biologically relevant and functional tissue constructs. To mimic the complexity of structures and compositions that characterize a tissue's own ECM, several ECM-extraction methodologies have been used. However, their outcomes seldomly reflect what is found in native tissue's ECM. Herein, we propose the use of supercritical carbon dioxide (scCO2) technology to extract the ECM components of cell-sheets that have shown promising results in creating accurate 3D microenvironments replicating the cell's own ECM, to be used in the preparation of bioinks.

Methodology
The ECM extraction protocol best fitted for cell sheets was defined considering efficient DNA removal with a minor effect on the ECM produced by cells. Cell sheets of human dermal fibroblasts (hDFbs) and adipose stem cells (hASCs), obtained as previously described (Cerqueira et al., 2013, 2014) were processed using a customised supercritical system, by varying the pressure of the reactor, presence, exposure time and type of co-solvent. Quantification of the amount of DNA, protein and sGAGs was carried out to determine the efficiency of the extraction in relation to standard decellularization methodologies. To develop ECM-based bioinks, the extracted ECM was combined with alginate as a support polymer. The influence of the alginate (1%, 2% w/vol) and ECM (0.5% and 1.5% w/vol) amount on the printability of the blends was addressed by analysing the rheological behaviour of the suspensions. Blends with suitable rheological performance were selected for cell encapsulation studies, to assess the influence of bioink composition on cell viability (calcein/Propidium iodide). Finally, 3D printed constructs were fabricated using an in-house built extrusion-based bioprinter, and the impact of the extrusion process on cells was assessed.

Results
The optimised scCO2 protocol allowed efficient removal of DNA while preserving a higher content on ECM proteins and sGAGs than the standard methodologies. Characterization of extract's composition also revealed that the ECM produced by hDFbs (fECM) and hASCs (aECM) are distinctively affected by the extraction protocols. Furthermore, rheological analysis indicated an increase in viscosity with increasing ECM composition, an effect even more prominent in samples containing aECM. 3D printing of alginate/ECM constructs demonstrated that cell viability was only marginally affected by the extrusion process, and this effect was also dependent on the ECM source.

Conclusions
Overall, this work highlights the benefit of supercritical fluid-based methods for ECM extraction and strengthens the relevance of ECM-derived bioinks in the development of printed tissue-like constructs.

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keywords: Extracellular Matrix, Supercritical CO2, Bioinks, 3D Bioprinting

62825415655

SYNTHESIS AND CHARACTERISATION OF FIBRIN-DEXTRAN HYDROGELS FOR THE APPLICATION IN TISSUE ENGINEERED HEART VALVE IMPLANTS

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Heart valve diseases have become one of the main causes for mortality worldwide. To increase the life expectancy of these patients, surgical replacements using mechanical or biological heart valve implants are most commonly used. However, mechanical implants entail the lifelong intake of anticoagulation medication and biological implants exhibit a limited lifetime of 10-20 years. In addition, these implants are limited by their inability to grow with the patient and repair themselves. Hence, the fabrication of tissue engineered heart valve implants that exhibit high mechanical stability while maintaining biocompatibility is strived for. For tissue engineering purposes, hydrogels are a promising candidate for encapsulating cells. In contrast to conventional hydrogels, fibrin hydrogels are auspicious as they are naturally occurring in the human body and provide a three-dimensional environment for cell proliferation and growth. An increase in the mechanical stability of fibrin hydrogels can be achieved through the addition of additives, such as linear reactive copolymers. However, the molecular weight of synthetic copolymers is limited and the long-term degradability and biocompatibility is unknown. Biopolymers such as dextran on the other hand, present a promising option, as they exhibit biocompatible and biodegradable properties combined with tunable molecular weights and affordable production cost.

In this work, we synthesize a hydrogel composed of natural fibrin and dextran hydrogels for the application in tissue engineered heart valve implants. For this purpose, dextran is functionalised with an allyl group using glycidyl methyl ether, which enables hydrogel formation via dithiol addition. The fibrin hydrogel was synthesized through the addition of thrombin and fibrinogen. By varying the concentration of the components and ratio between dextran and fibrin hydrogel, different compositions of fibrin-dextran hydrogels were obtained and analyzed using rheology, scanning electron- and confocal microscopy.

The gelation process and mechanical properties of the fibrin-dextran hydrogel were investigated using rheology. In comparison to the pure fibrin or dextran hydrogel, which only exhibit one gelation point, the rheological data of fibrin-dextran hydrogel mixtures suggests two independent gelation points. Furthermore, a higher storage modulus could be achieved for fibrin-dextran hydrogels in comparison to the pure fibrin hydrogel. The images obtained from Cryo-Scanning Electron Microscopy of the fibrin-dextran hydrogels show a combined structure occurring in typical fibrin and dextran hydrogels. The imaging of stained fibres using confocal

microscopy was successful. First experiments on the cell viability of fibrin-dextran hydrogels are currently being conducted.
To conclude, we generated a novel hydrogel composed of fibrin and dextran with excellent mechanical properties and biocompatibility, which shows promising behavior for application in tissue engineered heart valve implants.

keywords: hydrogel, fibrin, dextran, biomaterial, polysaccharide

41883639855

TISSUE ENGINEERING OF URETHRA – PREPARATION OF ACELLULAR SCAFFOLD

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Introduction: Several scaffolds have been introduced for urethral tissue engineering. However, acellular human urethral scaffold harvested from deceased donors may provide significant advantages in comparison to synthetic, composite or other biological scaffolds. The aim of this study was to develop a protocol for decellularization of human urethra that preserves substantial components of extracellular matrix (ECM), which are essential for subsequent recellularization mimicking the natural environment of the original ECM.

Methodology: 12 human urethras were procured from deceased donors. Part of every procured urethra was used as a control sample for analysis. The protocol design was enzyme-detergent based, 0.25% trypsin, 1% Triton X-100 supplemented with enzymatic removal of DNA residues was used. Subsequently, the scaffold was washed in distilled water for 7 days. The efficiency of decellularization was determined using histology examination, immunohistochemical staining, electron microscopy and DNA quantification.

Results: Histology confirmed cell removal and preservation of urethral structure after decellularization. The preservation of collagen I, III, IV, elastin and fibronectin was confirmed by histologic examination and immunohistochemical staining. Scanning electron microscopy confirmed maintenance of ultrastructural architecture of ECM, ground substance and fibers. DNA content in decellularized urethra was significantly lower compared to the native sample and the criteria for decellularized tissue were met.

Conclusion: This study demonstrates the feasibility of an enzyme-detergent-based decellularization protocol for the removal of cellular components and maintenance of urethral ECM and its ultrastructure. Results provide solid ground for recellularization and urethral tissue engineering which will follow.

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keywords: urethra, scaffold, decellularization, tissue engineering

PS17
Biomaterials, Stem Cells and Ostogenesis, Immunogenicity and Biocompatibility

31412702286

3D PRINTED IMMUNOMODULATORY SCAFFOLDS WITH CONTROLLED DRUG RELEASE FOR BONE REGENERATION

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Introduction: The immune system plays a vital role in inducing inflammation, tissue repairing and regeneration. Currently, there is an endeavour to investigate the role of macrophages in bone tissue regeneration. It has been elucidated that macrophages can polarise to proinflammatory M1 and anti-inflammatory M2 phenotypes. However, the role of macrophage phenotypes in bone tissue regeneration is not fully understood and needs more investigation. On the other hand, the interaction of macrophages with an implanted biomaterial is inevitable. It has been acknowledged that the property of implanted biomaterials may cause exacerbated immune response, which leads to the rejection of implants and bone tissue deterioration. Therefore, controlling the macrophage phenotypes by releasing immunomodulatory drugs from the implant for directing the bone tissue regeneration outcomes is the scope of this study.

Methodology: Polycaprolactone (PCL) polymer was alone or mixed with different ratios of poloxamers F127, F68, L31, and surfactants SPAN80 and emulsifying agent. Dexamethasone was added to polymer mixtures as 0.12% (w/w). Polymer inks were printed by a 3D Discovery printer (regenHU, Switzerland) with a dimension of 10 x 10 x 5 mm3. The release of Dexamethasone from 3D printed PCL scaffolds were conducted in PBS at 37^{0}C. Afterwards, the immunomodulatory effect of the released drug from 3D printed scaffolds on M1 polarization was evaluated by quantifying Tumour necrosis factor-alpha (TNF-α), IL-6, IL-1β, IL10, CCL-18 cytokines by DuoSet ELISA kit (R&D Systems); also surface markers calprotectin and mannose were stained.

Results: Scaffolds composed of emulsifying agent showed a prolonged release profile up to 30 days with the cumulative release of 80%, on the other hand, scaffolds made of poloxamer and surfactants showed 100% cumulative release of drug within 24h hrs whereas, PCL alone reached 100% of cumulative release in 7 days. The released dose of Dexamethasone from scaffold made of emulsifying agent and PCL alone was reduced the production of TNF-α, IL-6 and IL-1β from Polarized M1 macrophages. Also, it enhanced the expression of CCL-18 and Mannose receptor expression at day 7.

Conclusion: our result demonstrated a prolonged release profile of Dexamethasone from 3D printed PCL scaffolds composed of emulsifying agent compared to other scaffolds. The released dose of Dexamethasone modulated the immune response of macrophages without compromising its viability in vitro.

keywords: Immunomodulatory, 3D printing, cytokines, Bone, Macrophages

83767209909

A NOVEL IN VITRO STRATEGY FOR LONGER TERM DIFFERENTIATION OF HUMAN EMBRYONIC TISSUES AND SIMULATIONS OF TERATOMA FORMATION USING HUMAN PLURIPOTENT STEM CELLS

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Introduction:
Human pluripotent stem cells (hPSCs) are an alternative model for recapitulating key milestones of human embryogenesis during tissue development, which is an important and highly regulated process, yet poorly understood1. Currently, creating an in vitro model that provides the appropriate microenvironmental cues in a spatiotemporal manner to enable hPSCs to differentiate into complex 3D tissue structures remains a challenge2,3.

Methodology:
We have developed a novel three-dimensional in vitro system that has the ability to maintain and form complex tissue structures with recognisable features of various human embryonic tissues from the three germ layers by combining size controlled human embryoid bodies (hEBs) with subsequent maintenance and differentiation on a porous scaffold.

Results:
This approach extends culture time by flattening EB shape, resulting in reduced diffusion distances and increased cell viability, permitting longer term cultures, increased cell differentiation and the formation of high order structures. Current work focuses on directing the differentiation of hEB structures towards selected germ layers to study specific developmental pathways. Future work will involve the introduction of perfusion, another key microenvironmental parameter, in order to increase physiological relevance and promote even more complex tissue differentiation. Finally, human induced pluripotent stem cell populations will be used to fully validate and explore the capabilities of the model.

Conclusion:
We hypothesize that through the development and application of new in vitro technologies, it is feasible to direct the differentiation and extend the growth/maturation of tissues derived from hPSCs. We anticipate that this novel approach will provide an opportunity to study early stages of tissue development and deliver a controllable and reproducible animal-free alternative to assess the pluripotency of hPSCs.

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keywords: human pluripotent stem cells, embryoid bodies, porous scaffold, 3D

52354523886

A NOVEL NANOSTRUCTURED-MESOPOROUS-AMORPHOUS SILICA AND CALCIUM PHOSPHATE BIOMATERIAL FOR BONE REGENERATION APPLICATIONS

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Introduction
Autologous bone transplantation is the gold standard for reconstruction of large bone defects in the fields of orthopedics and odontology. However, limitations such as the grafting mass needed, its availability, and donor site morbidity has led to the search for osteo-inductive biomaterials that possess similar composition and structure to the main inorganic component of bone tissue. In our study, we utilized a bone marrow-derived mesenchymal stem cell (BMSCs) model in 3D to evaluate the osteogenic potential of a novel nanostructured-mesoporous-amorphous biomaterial candidate.

Methodology
Initial toxicological tests of the novel biomaterial were performed by Live/Dead cell viability assay at various concentrations of the biomaterial.
BMSCs from four healthy donors were mixed with collagen I to achieve 3D structures. These constructs were cultured for 3-5 weeks with or without osteogenic differentiation medium. The groups with no addition of biomaterials were set as negative controls. As positive control, the same setup was used with the addition of hydroxyapatite/β-tricalcium phosphate (HA/βTCP). Finally, our test group contained the novel biomaterial candidate instead.
Osteogenic specific staining (Alizarin red, Von Kossa, Osteoimage®) were used to detect the evidence of mineralization. Osteogenic specific gene expression was analysed by qRT-PCR.
In addition, 748 genes involved in various metabolic pathways were quantified by using a single molecule detection system based on fluorescent barcode-hybridization (nCounter®, NanoString).

Results
In this study we introduced an amorphous silica, SiO2, as an additional constituent to calcium phosphate material, thus obtaining a predominantly amorphous material with the composition of CaO-SiO2-P2O5.This material is nanostructured and mesoporous with an average pore size of 13 nm. Toxicology tests proved that the biomaterial is not toxic for BMSCs in concentrations below 10 mg/ml. Histological staining showed clear ossification of gels containing the novel biomaterial even in absence of differentiation medium. The calcification was also more homogeneous compared with the positive control: HA/βTCP. However, qRT-PCR data suggested higher levels of osteogenic related genes in the HA/βTCP group compared with the test group. Multivariate analysis of the 748 metabolic-related genes with nSolver software showed that the novel biomaterial in absence of differentiation medium induced different metabolic pathways compared to the pathways induced by HA/βTCP. No significant difference in gene expression was observed when the groups had been cultured in presence of differentiation medium. Taken together, our results indicate that the novel biomaterial tested in this study, on its own, can induce osteogenesis in BMSCs.

Conclusions
This study shows evidence that this novel nanostructured-mesoporous-amorphous biomaterial has a high potential as a candidate for bone regeneration applications due to its good osteo-inductive capabilities.

keywords: BMSCs, biomaterial, osteogenesis, osteoinduction, nanomaterial

41883659286

ALIGNED POLYURETHANE NANOFIBERS COATED WITH POLYPYRROLE: ANISOTROPY AND CONDUCTIVITY AS CELL-INSTRUCTIVE CUES

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Introduction
In recent years of regenerative medicine research, polymers have been utilized in an enormous number of types, variations and modifications, yet their potential is far from exhausted. One of the polymers with great variability of shapes and properties is polyurethane. It is a relatively cytocompatible polymer spinnable into nanofibers, allowing the natural extracellular matrix to be mimicked. Another cell-instructive cue of a biomaterial, which is especially advantageous for the cultivation of cardiac and neural tissues, is conductivity. Here, conductive polymers, such as polypyrrole, are very attractive thanks to their combined electron and ion-based conductivity as well as their quick and modifiable chemical synthesis.

Methodology
In this submitted research, electrospinning of polyurethane was used to prepare both anisotropic mats (aligned fibers) and isotropic mats (randomly oriented fibers). These substrates were next coated by in situ polymerization of conductive polypyrrole. Morphology of the created composites was characterized by atomic force microscopy and scanning electron microscopy. The impact of the two cell-instructive factors on cell behavior was studied by fluorescence microscopy of fibroblasts, and more importantly, of embryonic stem cells.

Results
Testing revealed decreased cytocompatibility of synthesized polypyrrole coatings which may be easily improved by beforehand adhesion of biomolecules. In addition, a significant change in morphology of fibroblasts, mainly initiated by the fiber alignment, was observed. Nevertheless, neither anisotropy nor conductivity affected the clustering of embryonic stem cells in the undifferentiated state.

Conclusion
Results of this research deepen current limited knowledge on the polymer biomaterials constructions, biomimicking modifications, and their impact on cell behaviors. However, the challenge still remains in comprehension of every factor which controls and affects cell cultivation, especially stem cells. Complete understanding would allow perfect scaffold designing for specific tissue growth achievement.

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keywords: Cytocompatibility, stem cells, biomimicking, in situ coating.

94238117226

APPLICATION OF CARTILAGE EXTRACELLULAR MATRIX FOR ENHANCING THE THERAPEUTIC EFFICACY OF RHEUMATOID ARTHRITIS DRUG

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation and intra-articular cell infiltration. Currently, RA is still incurable, and treatment is aimed at relieving symptoms. Therefore, RA patients must be taken drugs continuously. Methotrexate (MTX) has been used for the treatment of RA since the 1980s and is the most used drug among disease-modifying anti-rheumatic drugs (DMARDs). These drugs can delay the progression of RA by effectively inhibiting cell proliferation and inflammation. However, various side effects such as nausea and diarrhea exist and the treatment effect of the drug decreases when taken for a long time. So, it must take a high-dose drug or in combination with other DMARDs for the treatment of RA. Native cartilage is a representative avascular tissue and consists of mainly collagen and glycosaminoglycan. We extracted the porcine cartilage extracellular matrix (CECM). Many papers were known to various tissue-derived extracellular matrix can modulate inflammatory environment. So far, researchers have been applied tissue-derived extracellular matrix to the treatment for degenerative and immune diseases. The hypothesis is that low-dose MTX with CECM can have a synergistic therapeutic effect on the RA animal model. To prove it, this study aim was to evaluate the CECM as a treatment CECM biomaterial. The experimental group was divided with Normal, Inflammation, 88μM MTX (high-dose), 44μM MTX with CECM, 11μM MTX (low-dose) with CECM, 2% CECM alone. In the results, human synovial cell (SW 982) in the high-dose MTX group showed the cytotoxicity, but low-dose MTX with CECM and 2% CECM alone groups did not showed the cytotoxicity based on cell proliferation assay. Regarding immune cell response, low-dose MTX with CECM group showed the depression of mouse macrophage cell (RAW 264.7) activation and proliferation like high-dose MTX. In addition, expressions related inflammatory cytokine genes had been also suppressed by treatment, both high-dose MTX and low-dose MTX with CECM. In conclusion, the low-dose MTX with CECM showed similar therapeutic effects to the high-dose MTX. It means we could reduce the side effects of high concentration MTX currently used for RA treatment. We can expect that suggested drug supporting biomaterial using cartilage ECM can provide conveniences, such as reducing the dosing interval and drug volume to RA patients.

keywords: Rheumatoid Arthritis, Cartilage Extracellular Matrix, Methotrexate, Anti-inflammation

73296349455

CONDUCTING POLYANILINE FILMS PREPARED IN COLLOIDAL DISPERSION MODE IN PRESENCE OF BIOACTIVE POLYSACCHARIDES

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Introduction
First place of interaction between the biological and artificial system is the surface. Surface properties can influence many cellular functions. The efficient way of surface functionalization is thin composite films prepared in situ in colloidal dispersion mode. The combination of intrinsic electrical conductivity with easy preparation and modification procedures of polyaniline (PANI) opens the door for its promising applications.

Methodology
PANI coating films were prepared via the oxidation of aniline hydrochloride with ammonium peroxydisulfate where sodium hyaluronate (SH) or chitosan (CH) were used as stabilizers. Surface energy (measured by acid base method), surface topography (AFM), and conductivity (van der Pauw) of functionalized surfaces were determined. Biological properties such as determination of protein adsorption on the film, and adhesion of mouse embryonic fibroblast cell line NIH/3T3 were investigated. The antibacterial activity of the films against E. coli and S. aureus was also tested.

Results
The results of surface energy indicate that the addition of biopolymers (PANI-SH and PANI-CH) changed the surface energy. PANI films without stabilizers showed twice as high conductivity. Films with polysaccharides also showed antimicrobial activity against E. coli. Compared to the reference sample, protein adsorption increased only in case of unstabilized PANI and PANI-SH. Mouse fibroblast cells were capable of adhesion on PANI films stabilized by SH and CH.

Conclusion
PANI films itself do not provide an appropriate cell response. On the other hand, PANI films stabilized by SH and CH improve their biological properties. Therefore, the presence of bioactive polysaccharides enhances the applicability of PANI in tissue engineering and biomedicine.

Acknowledgments
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keywords: Polyaniline, Conducting films, Colloidal dispersions, Surface analysis, Cell compatibility

73296318484

DEVELOPMENT OF AN ANTI-INFLAMMATORY BIO-INK LOADED WITH CURCUMIN NANOPARTICLES FOR TISSUE ENGINEERING APPLICATIONS

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Curcumin is a well-known natural anti-inflammatory agent derived from turmeric, and it is commonly used as an herbal food supplement. Nowadays, the use of curcumin has also reached the biomedical and tissue engineering fields. To overcome the hydrophobicity of curcumin, nanoparticles (NPs) were synthesised using a solvent evaporation, oil-in-water emulsion method. The curcumin nanoparticles were incorporated into a polymer (polylactic acid - PLA), as polymeric nanoparticles can offer controlled drug release. The newly synthesized curcumin nanoparticles were analysed using a scanning electron microscope (SEM), where results showed the nanoparticles had a size ranging from 50-250nm. Three concentrations of the nanoparticles (0%, 0.5%, and 2%) were seeded onto NHI/3T3 fibroblast cells for the evaluation of cytotoxicity using Alamar Blue assay. Then, the curcumin NPs were incorporated into an alginate/gelatin-based solution, prior to crosslinking using a calcium chloride solution 200nM. Hydrogels were then characterised regarding their chemical, mechanical and rheological properties. Following hydrogel optimization, the hydrogel-loaded 2% curcumin NPs was selected as a bio-ink for 3D printing. The biological assessment of the hydrogel-doped 2% curcumin NPs was conducted using THP-1 cells, a human monocytic cell line. Cell viability and immunomodulation were evaluated using lactate dehydrogenase (LDH) and tumour necrosis factor alpha (TNF-α) enzyme-linked immunosorbent (ELISA) assay, respectively. Results show that the hydrogel-loaded 2% curcumin NPs was cytocompatible and supressed the production of TNF-α. In conclusion, curcumin NPs were successfully developed and incorporated to an alginate/gelatin bio-ink precursor for 3D printing applications. This novel bio-ink demonstrates desirable properties for the supresison of immune cell activation and inflammation, particularly interesting in the fabrication of tissue engineering constructs.

keywords: Hydrogel, Bioink, 3D printing, Curcumin, Nanoparticles

73296356799

ELASTIC POLYMERIC CAPSULES FOR OSMOSIS-DRIVEN VACCINE DELIVERY

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Introduction:
The development of vaccines represents one of the most impactful milestones in the improvement of global health over the last 200 years1. After a first administration of an antigen, the delivery of a booster is usually required to potentiate the immunostimulatory properties of the antigen2. Despite the undeniable success of vaccines, delivery could be improved. The use of controlled release technology could circumvent the need for multiple interventions for administering booster doses. In this project, osmotic pressure is exploited as a trigger for the spontaneous release of the booster from a subcutaneous polymeric capsule after a given lag time.

Methodology:
Implantable capsules made of crosslinked poly(ε-caprolactone) (xPCL), non-crosslinked PCL , and a crosslinked copolymer of ε-caprolactone and lactide were prepared by dip coating. Glucose was used as the osmotic agent and the food dye Brilliant blue FCF was employed to assess the osmosis-driven release kinetics. The devices were loaded with 70 µL of the glucose/dye stock solution, capped and incubated in PBS at 37 °C for up to 72 days in order to assess water uptake, and dye release through UV spectrophotometry. The cytotoxicity of the devices was evaluated by performing WST-1 and Alamar blue assays using macrophages and fibroblasts.

Results:
All capsules showed a gradual water uptake over several weeks, followed by a rupture which initiated a partial instant release followed by a trickle. The devices made of xPCL released a higher amount of payload at burst (10 %) than those made of PCL, which released only 1% of their total load. These results confirmed that a higher elastic recovery is required in order to increase the release at burst. The capsules made with the copolymer released the highest amount of payload at burst (62% of the total). This was due to the crosslinked and amorphous nature of the copolymer, which endows it with a higher elastic recovery in comparison with that of the semi-crystalline materials (PCL and xPCL). With regard to the toxicity of the materials, the results obtained in vitro showed that the viability of the cells grown on the polymers was comparable to that of cells grown on tissue culture polystyrene. Therefore, these materials did not cause cytotoxic responses on the cells used in the experiments performed.

Conclusions:
The results showed herein demonstrated the delayed burst release of a dye encapsulated in devices manufactured with a resorbable, non-cytotoxic polymer. The devices made of poly(-caprolactone-co-lactide) released approximately 6 to 60 times more payload at burst than the capsules made of xPCL or PCL respectively. The cytotoxicity studies performed in vitro showed that the materials used for the synthesis of the capsules did not cause toxic responses in the cells used for testing.

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keywords: Immunobioengineering, Biofabrication, Vaccine delivery

83767236655

EXPLORING THE EFFECT OF VISCOSITY ON OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS WITH CONTROLLED CELL MORPHOLOGY

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Introduction
Recently, researchers have found increasing evidence that along with other mechanical cues of the cellular microenvironment, viscosity also has an influence on stem cell fate. However, it is barely studied how cells sense the viscosity of microenvironment. In this study, we explored the effect of viscosity of culture medium on osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hMSCs). The cell morphology was precise controlled by micropatterns to compare how cell morphology and viscosity synergistically affect osteogenic differentiation.

Methodology
Micropatterns having circles of different size (30, 40, 60 and 80 μm diameter) and 60 μm diameter circle of different aspect ratio (1:1, 2:1, 4:1 and 8:1) were prepare on tissue culture polystyrene (TCPS) discs using photoreactive poly(vinyl alcohol)(PVA). The hMSCs were cultured on the micropatterns in viscous osteogenic induction media. The viscosity of the media was adjusted by adding 1wt% of PEG of different molecular weight. Actin filament was stained after hMSCs were cultured for 24 hours. To examine the osteogenic differentiation, alkaline phosphatase (ALP) staining was performed after culture for 2 weeks, and calcium deposition was stained by alizarin red s after culture for 3 weeks.

Results
The hMSCs cultured on the micropatterns attached to the circles and ellipses of micropatterns and their morphologies were well controlled by the micropattern structure. After 2 weeks culture in the induction media with different viscosity, the ratio of ALP positive cells was counted. For large cells (40, 60, and 80 μm), ALP-positive cells increased with increase of viscosity. Small cells (30μm) showed the same level of ALP activity in different viscosity. When hMSCs were cultured on the 60 μm circle and ellipse micropatterns with a fixed size but changing aspect ratio, the number of ALP-positive cells increased with increasing viscosity. High viscosity further enhanced the promotive effect. After 3 weeks culture. The calcium deposition staining showed the consistent result with that of ALP staining.

Discussion
These results indicated that viscosity of culture medium could affect osteogenic differentiation of large hMSCs more significantly than small hMSCs. The effect of high viscosity was enhanced by aspect ratio. Cell morphology and medium viscosity showed synergistical effects on osteogenic differentiation of hMSCS.

keywords: Viscosity, stem cells, osteogenic differentiation

20941808088

HYALURONIC ACID BASED NANOFIBROUS MATERIALS STABLE IN AQUAEOUS ENVIRONMENT FOR INCORPORATION OF ACTIVE SUBSTANCES

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Introduction
Nanofibrous materials have the potential to be used in medicine, the most mentioned areas being topical applications, e.g., skin wound healing. Promising materials are biopolymers such as hyaluronic acid (HA), which is a part of the extracellular matrix and plays an important role in the inflammatory and granulating phase of wound healing. Nevertheless, HA is highly hydrophilic and nanofibrous materials of HA dissolve in water immediately, which excludes the applications requiring longer degradation periods. This problem can be overcome by the covalent crosslinking of HA or hydrophobization by modifying the polymer chain.

Methodology
Nanofibrous materials based on a hydrophobized HA derivative – lauroyl hyaluronan (L-HA), a photo-cross-linkable derivative – furanyl hyaluronan (F-HA), or composites with different ratios of F-HA:L-HA were prepared by electrospinning. Materials containing F-HA were further crosslinked in a UV crosslinker for 60 minutes. The morphology of nanofibrous mats was studied by SEM in the dry state and after the immersion into phosphate buffer solution (PBS). The non-enzymatic and enzymatic degradation study was performed, respectively, in sodium acetate buffer (pH 5.3) containing bovine serum albumin (BSA) and in sodium acetate buffer with BSA and bovine testicular hyaluronidase. The effect of the nanofibrous mats' extracts on the viability of 3T3 fibroblasts and migration of HaCaT keratinocytes (scratch test) was assessed. NHDF cell adhesion on nanofibrous materials incubated in partially heparinized blood was also monitored. Active substances (octenidine dihydrochloride – OCT, triclosan - TRI) were incorporated into the composite materials. The release profiles and solubilization of the nanofibrous matrix were studied in PBS and PBS+BSA.

Results
The diameter of the prepared fibers ranged from 130 nm (F-HA) to 650 nm (L-HA). The nanofibrous structure was preserved even after 72 hours in PBS for materials with the equal ratio of F-HA:L-HA (C1), or with a majority of F-HA (C2), thus maintaining the porosity required for gas exchange, fluid drainage, etc. The nanofibers consisting mainly of L-HA eventually fused into a film. The materials from F-HA exhibited the fastest degradation, and the degradation rate decreased with decreasing ratio of F-HA in the sample. None of the extracts from the nanofibrous mats was cytotoxic, neither affected the keratinocyte migration. The NHDF cells adhered and proliferated on samples C1 and C2. TRI was released into PBS gradually, but burst release occurred in PBS+BSA. On the other hand, OCT was not released into PBS and released

gradually into PBS+BSA, simultaneously with the solubilization of the polymers.

Conclusion
Nanofibrous mats based on HA derivatives that are mechanically stable in aqueous environments were prepared by electrospinning. Materials gradually degraded in the media enriched with proteins and enzymes were not cytotoxic and did not influence cell migration. Cellular adhesion and proliferation were observed on mats preserving their fibrous structure in the wet state. Nanofibrous materials made of the combination of F-HA and L-HA were further modified by the incorporation of OCT or TRI. The release rate of the incorporated active substances depended on their physicochemical characteristics and dissolution medium.

keywords: hyaluronic acid, hyaluronan derivatives, nanofibers, electrospinning

62825469759

MAGNETIC IRON OXIDE NANOPARTICLES FOR THE DELIVERY OF THERMAL THERAPY FOR THE TREATMENT OF PRIMARY ALDOSTERONISM

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Introduction
To minimise damage to surrounding tissues, targeted delivery of therapeutics to the tumour is highly desirable, and the development of nanotechnology has shown promising results. Magnetic iron oxide nanoparticles (MIONPs) have been gaining traction over the years for applications such as drug delivery, molecular imaging and delivering hyperthermia for treatment of various cancers (1). MIONPs have great therapeutic potential as they can be produced in various sizes and shapes, with the ability to modify the surface by coating the nanoparticles. MIONPs have the ability to be activated by external magnetic field to generate heat and to cause hyperthermia (2).
Translationally, the delivery of thermal therapy offers an option for minimally invasive definitive treatment of primary aldosteronism, an endocrinopathy of aldosterone excess/dysregulation which represents the commonest secondary form of hypertension. In this study, MIONPs have been used at different concentrations to evaluate nanoparticle uptake and rate of uptake by adrenal cortical and endothelial cells, as well as gain understanding of the location of nanoparticles within the cell.

Methodolody
Magnetic iron oxide nanoparticles (MIONPs) were provided by University of Kansas. Adrenal Cortical cell-lines (MUC1, H295R and HAC15) and Endothelial cell-line (HUVEC) were used in this study. MIONPs were added at concentrations of 0.5, 5, 10, 20 and 50 µg/ml to the cells and incubated overnight. MIONP uptake efficiency, rate of uptake and cytotoxicity was assessed by Flow Cytometry. Confocal Microscopy was used to image the cells following MIONP incubation. Cellular proliferation was assessed by Xcelligence system and alamarBlue. Cellular respiration was assessed by "Seahorse" technology. MIONP location within the cells was assessed by transmission electron microscopy (TEM).

Results
Following overnight incubation with MIONPs, Flow Cytometry showed significant uptake by MUC1, HAC15 and HUVEC cells at 10 µg/ml MIONP concentration. Confocal and TEM images revealed MIONPs in the cytoplasm and in the vesicles for all cell types. Live Confocal imaging showed MIONP phagocytosis specific uptake by the HAC15 cells.

Conclusion
The data indicates that MIONP level of uptake and rate of uptake is cell and concentration dependent.

References:
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2. Wang (et al.), Beilstein Journal of Nanotechnology: 444-455, 2012.

keywords: Nanoparticles, MUC1, HAC15, H295R, HUVEC.

94238124219

OSTEOFORMATION POTENTIAL OF A NEW PYROPHOSPHATE-BASED GLASS IN CRITICAL-SIZE DEFECTS IN THE RAT CALVARIUM

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Background
The treatment of bone defects, in maxillofacial surgery and orthopedics, represents a major public health challenge. Autologous graft remains the “gold standard” treatment, but the availability of grafts is limited. Bioactive materials, such as silicate-based bioactive glass (Bioglass®), are among the bone substitutes the most widely used as alternatives, for their osteogenic potential but their resorption is limited. New glass compositions containing mainly pyrophosphate and orthophosphate entities have been elaborated by soft chemistry [1]. Their biological interest is linked to the control of the resorption rate (through the orthophosphate/ pyrophosphate ratio) by biochemical hydrolysis. The present study aimed to investigate the osteoformation potential, the resorption rate and the impact on the early bone healing process of a pyrophosphate-based glass with a ratio orthophosphate/ pyrophosphate =3 (NaPYG_030), using a critical-size model in rat calvaria.

Methodology
Circular 8 mm-diameter bone defects were created on calvaria of 12 weeks-old male Winstar rats. They were left empty, or filled either with a composition of pyrophosphate-based glasses, NaPYG_030 $(Ca^{2+})_{1,57} (Na^{+})_{0,14} (H^{+})_{0,05} (PO_4^{3-})_{0,67}(P_2O_7^{4-})_{0,33}] (H_2O)_{10,36}$ (n=6) or with silicate-based bioactive glass Bioglass® (n=6), used as control. The radiopacity was evaluated in vivo, using micro-CT analyses, performed immediately after surgery and on days, 15, 30, and 60. Histological analyses were performed after animal sacrifice at 30 and 60 days post-surgery. Tissue response, bone formation, and material resorption were assessed using non-decalcified histology. The expression levels of selected inflammation-, angiogenesis-, and bone-related genes, in the defect’s tissue were determined using RT-PCR after animal sacrifice at 7 and 14 days post-surgery.

Results
In vivo follow-up of the defect’s radiopacity volume (evidence for the residual implanted material and the newly formed bone volume) showed differences related to the bone substitute used. Compared to the Bioglass®-filled defects, the NaPYG_030 group exhibited a significant ($p < 0.05$) decrease[1] in radiopacity at all time points tested. Moreover, the study of the transcriptional profile of the genes showed that in the presence of NaPYG_030 the expression of TNFα, IL-6, and IL-8, ANG, PECAM and VWF as well as that of ALP, BGLAP, SP7 and Col1A1 was significantly decreased on the day 14 post-surgery. On day 30 and 60 post-surgery, the histological analysis

showed an absence of inflammation and/or foreign body reaction and newly-formed bone around the particles of both implanted materials. However, while the mineralized tissue was in close contact with the Bioglass® particles, a thin layer of unmineralized tissue was observed at the interphase NaPYG_030-newly formed bone, suggesting an ongoing resorption process.

Conclusions
In conclusion, the results obtained in the present study indicated the biological performance of new pyrophosphate-based glass in critical-size defects. The NaPyG-30 influenced the early critical events involved in bone regeneration by modulating specifically the expression of genes pertinent to inflammation, angiogenesis and osteogenesis, and exhibited both, efficient bone-forming capacity and resorbability making it an attractive bone substitute option to repair defects in the maxillofacial area.

[1] Mayen, L. et al., Acta Biomater.103,333-345 (2020).

keywords: Biomaterial, bone healing, in vivo, Osteogenesis, bioactive glass

20941823877

OSTEOGENIC POTENTIAL OF OVINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS STIMULATED WITH FGF-2 AND BMP-2 AND COMBINED WITH 3D-SCAFFOLD

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INTRODUCTION
Mesenchymal stem cells (MSCs) are able to differentiate into osteogenic lineage and promote bone regeneration. However, direct cells transplantation into damaged tissue is not efficient to cover large bone defects. This problem could be solved by biocompatible scaffold support. Therefore, bone tissue engineering constructs based on a biomaterial scaffold, MSCs and osteogenic cytokines are promising tool for bone regeneration [1]. The aim of this research was to evaluate the influence of FGF-2 and BMP-2 on osteogenic potential of ovine bone marrow derived-MSCs seeded onto HA/PCL-based scaffold.

METHODOLOGY
MSCs were isolated from sheep bone marrow (n=12) and treated with 100 ng/ml BMP-2 and/or 20 ng/ml FGF-2, following 7, 14 and 21 days of incubation. The effect of cytokines on osteogenic stimulation of MSCs was investigated by qPCR for osteogenic lineage markers: BMP-2, Runx2, Osterix (Osx), Collagen I (ColI), Osteocalcin (Ocl) and Osteopontin (Opn). Cells were then cultured on the HA/PCL-based scaffold in αMEM medium (control), and αMEM supplemented with BMP-2 and/or FGF-2. The effect of cytokines on morphology of cells growing on the scaffold was observed during 21 days of incubation. MTT assay was applied for cell viability, and the osteogenic differentiation potential was examined by Alizarin Red S staining and ALP activity.

RESULTS
Osteogenic gene profile of MSCs showed that cells cultured 14 days in the medium with FGF-2 and BMP-2 exhibited a higher level of BMP-2 gene expression than those cultured in the medium with only FGF-2 (RQ 3.97 vs. 2.34; $p<0.0001$). The mRNA expression level of Runx2 increased over time in the MSCs treated with FGF-2 and BMP-2 (RQ 1.84 vs. 6.78; $p<0.0001$). The highest peak in Osx gene expression was observed on day 14 in the MSCs cultured with FGF-2 and BMP-2 (RQ 3.33; $p<0.0001$), whereas in ColI gene expression after 21 days of incubation with FGF-2 and BMP-2 (RQ 5.19; $p<0.0001$). The gene expression of the late osteogenic marker Ocl and Opn increased over time for MSCs treated with FGF-2 and BMP-2 ($p<0.0001$). Morphology of cells growing on the scaffold changed during 21 days of incubation. FGF-2 and BMP-2 stimulation enhanced capacity of MSCs adherence to the scaffold. Cells seeded onto scaffold and treated only with FGF-2 had grater proliferation rate than untreated cells or treated with both BMP-2 and FGF-2 ($p<0.005$). MSCs supported with both BMP-2 and FGF-2 and seeded on the scaffold enhance bone mineralization compare to the control as confirmed by ALP activity assay (absorbance at 405 nm 0.75 vs. 1.01; $p<0.005$) and Alizarin Red S staining (absorbance at 405 nm 1.40 vs. 2.56; $p<0.0001$).

CONCLUSIONS
FGF-2 and BMP-2 enhance osteogenic potential of ovine MSCs as confirmed by upregulation of mRNA for early (BMP-2, Runx2, Osx) and late (ColI, Ocl, Opn) osteogenic differentiation genes. MSCs proliferate well on the HA/PCL-based scaffold. FGF-2 stimulates MSCs proliferation, whereas BMP-2 improves their osteogenic potential. Thus, bone tissue engineering construct, comprised of HA/PCL-based scaffold, sheep bone marrow-derived MSCs, and cytokines may be potentially used for bone regeneration.

1. Stamnitz, S., Klimczak, A., Cells 10(8), 1925 (2021)

keywords: bone marrow MSCs, biomaterials, bone tissue engineering, osteogenic differentiation

62825446907

PATTERNED HYDROGELS WITH SPATIALLY TUNABLE BIOPHYSICAL AND BIOCHEMICAL PROPERTIES TO GUIDE 3D STEM CELL RESPONSE AND OSTEOGENESIS

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Structurally patterned materials offer various options for guided cell behavior. Cell behaviour can be guided by patterning spatially discretized biochemical, topological or mechanical properties or combinations thereof. Patterned materials allow to integrate multiple characteristics in single materials, resembling an anisotropy found in endogenous tissue regeneration. Previous studies using orthogonal Diels-Alder and thiol-ene crosslinking in alginate hydrogels showed that patterns in 2D affect cell attachment and differentiation. However, to employ the full potential of pattern principles a 3D cell encapsulation in such materials appears mandatory. Therefore, this study aims to evaluate cell responses in 3D alginate hydrogels with spatial patterns using biophysical and biochemical patterning characteristics, which aim to have applications in regenerative medicine.

Single-phase materials were formed using norbornene (N) and tetrazine (T) modified alginate (Diels-Alder reaction, spontaneous non-UV) together with matrix metalloproteinase (MMP) sensitive peptides as a degradable crosslinkers (thiol-ene reaction, UV). The variations in the N-T ratio and the concentration of MMP sensitive peptide crosslinker can tune the mechanical properties and degradability of the material. The patterns on the material were formed using a photomask with UV irradiation which allowed the MMPsens peptides bonds form in the UV regions and the N-T bonds form in the covered stripes. The materials were mechanically characterized using rheology and microindentation. Mouse embryonic fibroblasts (MEFs) were encapsulated in 3D and cell viability (live/dead staining), cell morphology (DAPI/phalloidin) and proliferative state (Ki67) were evaluated at days 1, 7 and 14.

The rheology of single-phase materials revealed similar elastic modulus of ~3kPa in the degradable (UV) and ~2kPa in the non-degradable (non-UV) materials. These results were comparable to the microindentation of patterned hydrogels, showing stripes with soft (~1.8kPa) and stiff regions (~3kPa). 3D encapsulation of mouse embryonic fibroblasts (MEFs) in single-phase and patterned hydrogels showed high viability (>85%) over the 14 days and a significant increase in cell number in degradable materials compared to non degradable. Cell morphology showed a significant increase in cell area and decrease in circularity indicating cell spreading in degradable hydrogels, whereas the cells in non-degradable materials remained round. The main differences in cell morphology were observed in filopodia formation, with significantly higher cell filopodia number and length on softer areas while they were reduced in stiffer ones. The proliferation marker Ki67 was highly expressed in softer materials compared to stiff ones.

The hydrogels showed spatially tunable mechanical and degradation characteristics in 3D that were determined by the crosslinking type. Such differences influenced the morphology and proliferation of MEFs. Further research will look at 3D encapsulation of human mesenchymal stroma cells in patterned materials with the aim to guide cell differentiation.

keywords: patterned materials, differentiation hMSCs, cell response

31412751247

PREPARATION OF FOLIC ACID-FUNCTIONALIZED GOLD NANOPARTICLES-GELATIN COMPOSITES SCAFFOLDS FOR ABLATING BREAST CANCER CELLS

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Introduction
Breast cancer is one of the major lethal diseases. Surgery has been widely used to treat breast cancer. But surgery cannot resect all cancer cells in some cases, which may result in cancer recurrence. Recently, photothermal therapy, which is based on photothermal ablation of cancer cells, has been developed as a promising cancer therapeutic strategy. Until now, various photothermal conversion agents such as gold nanoparticles (AuNPs) have been explored. However, poor targeting capability and low accumulation of nanoparticles in cancer tissues need improving. To increase the accumulation of nanoparticles, AuNPs have been immobilized in 3D porous scaffolds for repeated heating and local photothermal therapy.
In this study, folic acid (FA), a frequently used targeting ligand for cancer cells enriched with FA receptors, was introduced into the composite porous scaffolds to enhance cancer cells' capture ability. The photothermal ablation ability of the composite scaffolds was investigated both in vitro and in vivo.

Experiments
Firstly, the FA-gelatin conjugate was synthesized based on a reaction between folic acid (FA) and gelatin. Secondly, Gold nanoparticles (AuNPs) with different shapes (nanorod, nanostar) and different sizes (around 40.0 nm, 70.0 nm, and 110.0 nm) were synthesized by a seed growth method. The one-pot method was used for the synthesis of Au nanorods with a longitudinal length of 110 nm. Finally, the ice particulates porogen method was used to prepare FA-functionalized composite porous scaffolds embedded with FA-gelatin-coated AuNPs with different shapes and sizes.

Results and Discussion
The UV-VIS spectra indicated the FA-gelatin conjugate was synthesized and their conjugate rate was calculated and optimized. The TEM images showed the morphology and size of synthesized AuNPs. The UV-VIS spectra of AuNPs showed AuNR possessed two LSPR peaks and AuNS had an abroad LSPR peak, which might be useful for photothermal therapy.
The SEM images showed the composite scaffolds had spherical large micropores with good interconnectivity. The photothermal conversion curves showed photothermal-induced temperature changes of composite scaffolds was modulated with the different shape and size of embedded AuNPs. The FA introduced in composite scaffolds showed high tumor cell capture ability compared with control groups. The photothermal ablation efficiency of tumor cells was dependent on the photothermal performance of the composite scaffolds. The results indicated FA-functionalized AuNRs 70-gelatin composite scaffolds had a good tumor cell capture and photothermal ablation ability. For the in vivo experiments, temperature monitor during laser irradiated implantation site of mice showed that the temperature of implanted scaffolds without AuNPs was kept almost unchanged. In contrast, the temperature of composite scaffolds embedded with AuNPs was increased significantly. Besides, whole-body bioluminescence imaging showed the in vivo tumor cell ablation effect.

keywords: photothermal therapy, folic acid, composite scaffolds, gold nanoparticles, cell targeting

73296376149

SCAFFOLD-FREE INTERCONNECTED TOROIDAL TISSUE SHEETS FOR VARIOUS TISSUE ENGINEERING APPROACHES

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Tissue engineering significantly requires fresh approaches to achieve hierarchy of the targeted tissues in 3D forms where the feature size is not limited by diffusion. We are investigating interconnected toroidal tissue sheets made of MSCs co-cultured fibroblasts or ECs and utilizing them as building blocks for the required tissue architectures. A simple 3D printed PLA mould having the negative template was utilized to make a positive template over agarose in a tissue culture plate followed by seeding cells within them. Harvested tissue sheets after 72 hours were stable, posing high viability compared to the control 3D spheroids. Tissue sheets made of cocultured MSCs and L929 fibroblasts had significant tensile strength and were further rolled to form tubular architectures. As expected, cells in the rolled tissue sheet started migrating and enveloped to form tubular tissue structure with lumen. These interconnected toroidal tissue sheets can be employed as building blocks for regenerating complex tissue structures involving multiple layers, tubular architectures or together.

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keywords: Scaffold-free, toroidal tissue, 3D, hierarchy

73296376869

THE DIFFERENT EXPRESSION OF CYTOKERATIN 14 AND SONIC HEDGEHOG SIGNALING MOLECULE BY PORCINE HOLOCLONE-, MEROCLONE- AND PARACLONE- LIKE BUCCAL EPITHELIAL CELLS

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Holoclone-, meroclone- and paraclone-like colonies were identified in in vitro cultures of normal epithelial cells. In the present study we tested whether cytokeratin 14 (ck14) and sonic hedgehog (shh) expression profiles are correlated with three buccal epithelial clone types. Buccal epithelial cells were isolated from porcine buccal tissues obtained from 9 donors. Cells were seeded into 12-well cell culture plates with glass coverslips in density of 2x104 cells/cm2. Three different clone types: holoclones, meroclones and paraclones were identified by two independent investigators based on their morphology. To characterize and compare the clones the immunofluorescent stains for ck14 and shh were performed. Fluorescence intensity of individual cells, cell surface and fluorescence intensity of background were measured to calculate relative cell fluorescence (RCF). The strongest expression of ck14 and shh was observed in the holoclones (201,4±78,52 and 9,23±3,26 respectively; $p<0,05$). The comparable ck14 expression was observed in the mero- and paraclones ($p>0,01$). Meroclones expressed significantly lower levels of shh (2,52±0,94) compared to paraclones (4,31±1,3; $p<0,001$). This study showed that three buccal epithelial clonal types with differing properties can be isolated from buccal tissues. Holoclones are the richest in shh (+) stem cells and this cell population should appear to be a promising alternative for obtaining urothelial cells from external urinary bladder sources.

keywords: holoclones, meroclones, paraclones

41883638406

THE EFFECT OF ZINC IONS IN POLYMER-CALCIUM PHOSPHATE COMPOSITE SCAFFOLDS ON OSTEOGENIC DIFFERENTIATION OF HMSCS

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INTRODUCTION
The inorganic phase of bone contains various bioinorganic compounds, apart from calcium, phosphate and carbonate, such as zinc, copper, magnesium, sodium, strontium and many others. These elements have been shown to affect bone mineral features, such as crystallinity, mechanical properties, and degradation. Among these compounds, zinc is an essential element of various enzymes and proteins, for instance, alkaline phosphatase (ALP), lactate dehydrogenase, carbonic anhydrase, and transcription factors. Osteoinductive biomaterials triggering osteogenic differentiation of bone marrow-derived human mesenchymal stromal cells (hMSCs), achieved by the addition of growth factors or bioinorganics, are especially important in the treatment of critical-sized bone defects. In this study, we propose the use of a novel bioactive composite with high ceramic content composed of poly(ethyleneoxide terephthalate)/poly(butylene terephthalate) (1000PEOT70PBT30, PolyActive, PA) and 50% beta-tricalcium phosphate (β-TCP) with the addition of zinc in a form of a coating of the β-TCP particles. We hypothesise that the addition of zinc to the β-TCP will result in enhanced osteogenic properties.

METHODOLOGY
To investigate the effect of zinc on osteogenic differentiation of hMSCs, β-TCP particles were coated by zinc by immersing them in a zinc ion solution with a concentration of 15 or 45mM before additive manufacture of porous 3D scaffolds composed of 1000PEOT70PBT30 and β-TCP in 1:1 ratio. Before in vitro testing, 1000PEOT70PBT30- β-TCP, 1000PEOT70PBT30- β-TCP +Zn15 and 1000PEOT70PBT30- β-TCP +Zn45 composites were sterilised and seeded with hMSCs. Osteogenic properties were evaluated based on the results of DNA content, ALP activity and osteogenic gene expression on days 3, 7, 14 and 28.

RESULTS
In vitro assessment of the osteogenic properties of the porous 3D composite scaffolds showed similar proliferation and ALP activity of hMSCS regardless of the type of scaffolds they were cultured on.
The addition of zinc to the composite resulted in increased expression of ALP, collagen I,

osteocalcin and osteopontin at day 3 of culture. Furthermore, a comparison between the two concentrations of zinc showed higher expression of collagen I and osteocalcin on scaffolds coated by immersion in a 15mM zinc ion solution and higher ALP expression in the case of 45mM.
Regarding later time points of cell culture, the expression of collagen I was the highest on composites with 45mM of zinc on both days 14 and 28. Furthermore, the expression of osteocalcin and osteopontin were increased on day 14. On day 28 of culture, the presence of both concentrations of zinc resulted in increased collagen I expression in a concertation dependent manner and downregulation of osteocalcin and osteopontin expression.

CONCLUSION
The addition of zinc ions to 3D porous additive manufactured 1000PEOT70PBT30- β-TCP composites resulted in increased expression of several osteogenic markers. Interestingly, results showed that the expression of late osteogenic markers such as osteocalcin and osteopontin was upregulated at day 3 when cells were cultured on composite scaffolds containing zinc.

keywords: Bone regeneration, Zinc, Composite scaffolds, Calcium Phosphate Ceramics

83767214046

USING MULTIPLE SURFACE TREATMENT PROCESS TO REGULATE THE OSTEOGENESIS AND OSTEOCLASTOGENESIS OF TITANIUM SURFACE

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As the global elderly population increases year by year, the demand for dental implants also increases significantly, Titanium (Ti) is widely used in dental implants due to its good mechanical properties, biocompatibility and corrosion resistance. However, titanium implant surfaces are biologically inert, it won't react with cells actively and the implant may failed. The success of dental implants is highly associated with the osseointegration between the implant and the surrounding bone tissue, and the balanced osteoblast/osteoclast reaction is one of the key points during this process. However, with an increase in age, an imbalanced osteogenesis/osteoclastogenesis may occur, which will prolong the bone healing time and then affect the osseointegration of implants. This study intends to develop a multiple surface treatment process applied for dental implant application. So how to reduce its bioinertness and regulating the osteogenesis and osteoclastogenesis through surface treatment will be the important topic of this study. First is using the sand-blasting with large grit and acid-etching (SA), the most commonly used treatment in clinical dental titanium (Ti) implants. Second is using alkaline treatment to produce the micro/nano-scale network structure (SAA). In order to decrease the activity of osteoclast and enhance osteogenesis, the final step is using a natural cross-linker, epigallocatechin_-3-gallate (EGCG), to immobilized type I collagen on the surface of SLA-A (SAAEC). The results indicate that the multiple surface treatment shows micro/submicro/nano-scale network structure and it has good cell responses, such as osteogensis cell adhesion, proliferation and mineralization. Moreover, the effects of the multiple surface treatment processes on osteoclastogenesis shows inhibition. The multiple surface treatment process in this study has the same osteogenic response as the clinical commercial sandblasting/acid etching treatment, and also has the effect of inhibiting the osteoclastogensis. In the future, it is still necessary to analyze the mechanism of the multiple surface treatment process on the effect of osteogenesis and osteoclastogensis through co-culture and gene expression.

keywords: titanium, surface treatment, type I collagen, epigallocatechin-3-gallate, cell response

PS19
Biomimetic Approaches to Cardiovascular Regeneration: how and why?

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CAN STRUCTURAL AND BIOACTIVITY GRADIENTS MITIGATE INTIMA HYPERPLASIA ON A SMALL DIAMETER TISSUE ENGINEERED VASCULAR GRAFT?

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Small-diameter synthetic vascular grafts do not recapitulate native blood vessel's structural heterogeneity, which in the case of arteries, is based on the morphologically distinct tunica intima, media, and adventitia. These layers differ in composition and as a result in their specific function [1].

It can be then speculated that when one of these structures is neglected in the design of a device for coronary artery bypass, arteriovenous fistulae, or dialysis access a corresponding function will be impaired. For example, intima hyperplasia is one of the most common mechanisms of failure in commercially available vascular grafts, and it can be attributed to the absence of a tunica intima or to the absence of an adequate artificial structure within the graft able to fulfill an equivalent function [2].

The excessive proliferation of smooth-muscle-cells (SMCs) in artificial graft generally leads to a decrease in lumen patency, vessels obstruction, and thrombus formation. Multiple other factors can aggravate this process such as a mechanical compliance mismatch between the native tissue, and the graft as well as altered hemodynamic conditions that are in turn dictated by non-physiological wall shear stress.
This study proposes a three-layered bio-inspired scaffold designed to prevent hyperplasia by introducing the notion of structural and bioactivity gradients in tissue engineering small-diameter vascular graft (TEVG).

Biomimicry of morphological characteristics of the tissue, such as inner wall thicknesses, inner diameter, outer diameter, was based on scanning electron microscopy (SEM) of explanted porcine coronary arteries (N=3).
Next, two bio-processing techniques were utilized to create three morphologically different layers: thermally induced phase separation (TIPS), and electrospinning (ES). The tunica media was reproduced as a bioactive layer composed of decellularized small intestinal submucosa ExtraCellular Matrix gel (ECM) seeded with rat SMCs. While the adventitia and intima layers were produced by using poly(ester urethane)urea proceeded by TIPS and ES due to its biocompatible and biodegradable properties.

Four different configurations were fabricated for TEVG scaffolds where the three layers combination were permutated as follow: ES-ECM-TIPS; ES-ECM-ES; TIPS-ECM-TIPS; TIPS-ECM-ES. Each TEVG was tested under dynamic conditions using a pulsatile custom-made bioreactor. Histological analysis was conducted to quantify SMCs distribution per layer (cells/unit area) and cell migration (7 days).

After 7 days of dynamic culture, preliminary histological analysis confirmed the hypothesis that the cell-seeded in the central portion of the graft migrated towards the most porous graft layer (TIPS) suggesting that a structural and bioactive gradient can mitigate the intima overgrowth.

In the three-layer TEVG, with the TIPS-ECM-ES configuration, showed that the electrospun fibrous structure can act as a barrier [3] limiting cell infiltration into the intima. This in conjunction with a more penetrable layer (TIPS) can lead to tailored cell migration.

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keywords: Tissue Engineered Vascular Graft (TEVG), bio-fabrication, bioinspired scaffold, cell migration

31412740105

POLYESTERS BASED ON CITRIC ACID AND DIOLS WITH ANTIOXIDATIVE PROPERTIES AS VERSATILE MATERIALS FOR SOFT TISSUE ENGINEERING: STUDIES ON DEGRADATION AND CYTOCOMPATIBILITY

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Introduction
Poly(alkylene citrate)s – (PACs) are widely studied as candidates to produce temporary medical devices such as blood vessel prostheses, wound dressings, drug delivery systems, scaffolds, and matrices for tissue engineering. The advantages of PACs are susceptibility to hydrolytic degradation and the possibility of modification with free radical scavengers, which are known to tune inflammation and thus the tissue healing process. Glutathione (GSH) is an excellent antioxidant, efficiently scavenging of a wide variety of free radicals. The aim of this study was to find out if the chemical composition of PACs (GSH content, type of diol) influences the degradation kinetics and cytocompatibility of the resulting polymers.

Methodology
Two PACs: cross-linked poly(hexamethylene citrate) (cPHC) and poly(octamethylene citrate) (cPOC) with a molar ratio of citric acid to 1,6-hexanediol/1,8-octanediol equal to 2:3 without or with 0.4% or 0.8% GSH were synthesised [1]. For degradation studies, the samples were incubated in purified water at 37 oC up to 3 months. Then, the samples were weighed in a wet state and after freeze drying to evaluate weight loss and water absorption capacity, respectively. The hardness was assessed using Shore A method to evaluate the decrease in cross-linking density. Moreover, the pH of the incubation fluid was measured to study the degradation progress, while mass spectrometry was used to determine degradation products. In vitro cytocompatibility study of the PACs was performed using L929 fibroblasts, which were cultured in the 10% extracts of cPHC and cPOC samples with or without GSH. After 24 h and 72 h of culture, the Alamar blue metabolic activity and live-dead tests were performed.

Results
All PACs lost transparency with extending incubation time; the increase in swelling, viscosity, and adhesiveness of the samples were all observed. Furthermore, the GSH-modified samples lost their brown coloration as a function of incubation time. The weight loss was higher for cPHC than in case of cPOC samples which was attributed to higher hydrophilicity of materials prepared from shorter chain diol. Analysis of the mass spectra of the supernatants after the degradation process showed that small amounts of polymer chain fragments were released during the first 3 weeks of incubation and then the intensity of these characteristic peaks increased. The unit of citric acid appeared sporadically and in small amounts in the initial stages of degradation, although its intensity increased sharply from the the 5th week (cPHC) and from 8th week (cPOC), which, in turn, proves that cPOC samples degrade more slowly.

Conclusions
Performed studies show that degradation of POC is slow and homogeneous, which is promising

in terms of reducing the body's immune response. Biological studies with L929 cells did not show cytotoxicity of the materials, which indicates the right direction for the use of GSH to improve the biocompatibility of citric acid polyesters.

Acknowledgements
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keywords: degradable polymers, poly(alkylene citrate)s, glutathione, cytocompatibility,

52354506488

PREPARATION OF AUTOLOGOUS CARDIOMYOBLAST SHEET BY A NOVEL CELL SHEET ENGINEERING AND APPLIED IT TO TREAT ISCHEMIC CARDIOMYOPATHY

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From 2020's WHO data, Ischemic heart disease is already the world's leading cause of death, and it also brings a high burden to the world. Nowadays, almost all clinical therapies based on supportive therapies, not is curative therapies, which cannot regenerate myocardial tissue. If using heart transplantation, there has not been resolved yet that donated hearts will be little supply and the problem of high mortality. Moreover, cell therapy has become an emerging direction for myocardial regeneration in recent years, which uses a thermo-sensitive polymer pNIPAAm as a cultural matrix. Furthermore, Japan's medical market already has a local team listed its cell therapy product. On the other hand, we have been committed to developing new cell sheet engineering technology for regenerative medicine for a long time. In our design for myocardial cell sheet, the plasma-activated surface chemical modification was used to graft the combination of disulfide bond-containing amino acid and biopolymer of cell feed onto a porous and transparent PET membrane be a culture matrix. Furthermore, spontaneously reductive cleaving the disulfide bond with a reducing amino acid addition can achieve the cell sheet detachment mechanism. Briefly, after surface chemical modification, the contact angle measurement and Electron Spectroscopy for Chemical Analysis (ESCA) were used to confirm the change in the chemical structure of grafted molecules in the disulfide bond cut period in this study. Then, it was successfully prepared to a culture insert as a culture device by ultrasonic welding between PET membrane and culture insert frame, and then the myocardium and leg muscles for the reconstruction of myocardial tissue were cultivated. As far as the results are concerned, we have isolated and amplified myocardial and skeletal muscle progenitor cells from New Zealand white rabbits successfully prepared cell sheets of these two tissues and confirmed the correctness of the obtained cell sheets by immunocytochemical staining. It can say that we have confirmed that the new system for cell sheet engineering is feasible. We will use New Zealand white rabbits with chronic Ischemic cardiomyopathy (ICM) formed by an Ameroid constrictor and then reconstruct its myocardium with an autologous myocardial or skeletal muscle precursor cell sheet. We hoped to be applied to clinical myocardial reconstruction as soon as possible in the near few years.

keywords: Ischemic Cardiomyopathy, Cell Sheet Engineering, Regenerative Medicine, curative therapy

PS20
Biomimetic in vitro models for bone regeneration and cancer pathologies

73296307357

BIOENGINEERING TUMOUR STROMA TO MIMIC BONE-TUMOUR INTERACTION

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INTRODUCTION
Ameloblastoma (AM) is a benign, epithelial tumour of the jawbones originating from the residual epithelium of the tooth germ. AM shows a locally aggressive behaviour characterised by progressive bone resorption. The precise molecular mechanisms behind the disruption of bone homeostasis are unclear. We developed a novel 3D model composed of an AM tumour mass and a bone stroma to recreate the tumour microenvironment.

METHODS
Type I collagen was used to fabricate 3D models. The collagen/cell mix was plastic compressed using Lonza RAFTTM 3D cell culture system using AM-1 (plexiform ameloblastoma) and AM-3 (follicular ameloblastoma). We engineered an active bone forming stroma using primary osteoblasts in stiff collagen matrix. The gene analysis was completed through reverse transcriptase- quantitative polymerase chain reaction (RT-qPCR) and protein levels were measured using Enzyme-Linked Immunoabsorbent Assay (ELISA). The bone nodules formed in the bone-stroma were characterised by nano-computed tomography (CT), transmission electron microscopy (TEM), Raman spectroscopy and gene analysis. The images were analysed using Fiji ImageJ and statistical analyses were performed using GraphPad Prism with $p < 0.05$ was considered as statistical significance.

RESULTS
AM cells expressed higher levels of invasion and bone resorption markers in 3D compared to 2D. Matrix-metalloproteinase (MMP)-2 was 2-times upregulated in 3D ($p = 0.03$) and MMP-9 was 3-fold upregulated in 3D. The bone nodules had an average surface area of 0.1 mm2 and average height of 92.37 ± 7.96 mm over 21 days in 3D. Our 3D biomimetic model presented a woven bone phenotype with mineral and matrix components. When the gene correlation between bone forming 3D stroma and AM introduced 3D bone stroma, we observed downregulation of bone formation genes such as RUNX2. AM cells inhibited osteoblasts from forming new bone nodules and limited the growth of existing bone nodules.

CONCLUSION & DISCUSSION
The biomimetic 3D tumouroid model accurately mimicked native subtype cell morphology. A novel bone-stroma model was established and used to study the AM tumour microenvironment. This study is the first to report that AM cells inhibit bone nodule formation by osteoblasts. It paves the way to further studies exploring the pathways of crosstalk between AM cells and the surrounding bone microenvironment. The model could represent a unique opportunity to test the effects of medications upon bone formation and resorption subject to perturbation by AM.

ACKNOWLEDGEMENTS
Dr. Liebert Parreiras Noguiera performed the Nano-CT scans. Dr. Mark Turmaine conducted TEM sample preparation and imaging at University College London.

keywords: bone, tissue engineering, nano-ct, tumour storm

73296389649

DEVELOPMENT OF OSTEOSARCOMA 3D IN VITRO MODEL COMPRISING BONE-MIMICKING SCAFFOLDS AND A BIOMIMETIC BIOREACTOR

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INTRODUCTION
Cancers are one of the leading causes of death worldwide. Failure to obtain effective treatment could be attributed to the irrelevancy of commonly utilized two-dimensional (2D) in vitro and animal models in drug evaluation. Three-dimensional (3D) in vitro models aim to provide more resemblance to in vivo cancer cell microenvironment and therefore represent promising tools for reliable anticancer drug screening. Here, we present the development of a 3D in vitro model mimicking in vivo osteosarcoma cell microenvironment regarding extracellular matrix and hydrodynamic conditions by utilising bone-mimicking macroporous composite alginate scaffolds in conjunction with perfusion biomimetic bioreactor.

METHODOLOGY
Macroporous composite alginate scaffolds with incorporated mineral powder (2 wt% alginate, 2 wt% hydroxyapatite) were produced as previously described1. Scaffolds in dry and rehydrated forms (~10 mm in diameter, ~4.5 mm thick) were analyzed regarding porosity and the average pore size. The optimal cell seeding method was based on partial rehydration of the scaffolds in the cell culture medium, followed by manual seeding with K7M2-wt osteosarcoma cells (ATCC® CRL-2836™) at the cell density of 15x106 cells cm-3 of the scaffold volume. The cell-seeded scaffolds were then cultivated for 24 h under static conditions followed by cultivation in biomimetic perfusion bioreactors (“3D Perfuse”, Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) under continuous medium flow (0.27 cm3 min-1; superficial medium velocity of 40 μm s-1) for the next 7 days. Static 3D cultures served as controls. Cell viability was investigated by the MTT test, while the cell morphology and distribution within the scaffolds were analyzed by field-emission scanning electron microscopy (FE-SEM, MIRA 3 XMU instrument, TESCAN, Brno, Czech Republic) and histological staining (H&E) of scaffold cross-sections.

RESULTS
Biocompatible macroporous alginate scaffolds had an average porosity of 60%, which falls within the porosity range of trabecular bone2. Efficiency of the cell seeding method was above 80% while the cultivation under medium flow had negligible effects on the cell loss. MTT test confirmed that the cells remained viable and even more metabolically active in perfusion cultures compared to static conditions (control). Moreover, the cells retrieved from the scaffolds were able to attach to the cell culture plastic and proliferate over 48 h in 2D in vitro model. The FE-SEM analysis revealed the presence of cell aggregates attached to the pores. In addition, osteosarcoma cells spontaneously formed spheroids within scaffold pores confirmed by histological examination.

CONCLUSION
In this study, a 3D model for osteosarcoma cell culture was developed by using murine cells, macroporous composite alginate-based scaffolds and perfusion bioreactors. The cells attached to the scaffold interiors and spontaneously formed spheroids during a 7-day culture. Perfusion had positive effects on the cells indicated by the increased metabolic activity as compared to static controls. It can be suggested that this developed 3D model has the potential for utilisation in osteosarcoma drug testing, which is the next phase of model evaluation.

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keywords: cancer modeling, macroporous alginate scaffolds, perfusion bioreactors

62825407655

ENGINEERING OF STANDARDIZED HEMATOPOIETIC STEM CELL NICHES TO MODEL HUMAN HEMATOPOIESIS USING INDUCED PLURIPOTENT STEM CELLS

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Introduction
Hematopoietic stem cell and progenitor cell (HSPC) niches are provided by the bone marrow (BM) tissue, from where HSPCs ensure the blood cells production throughout individual's life. In contrast to the murine counterparts, human HSPC niches remain poorly understood due to accessibility and ethical difficulties. In order to bypass these limitations, our lab has engineered different 3D in vitro models of human BM niches combining patient-derived cells, scaffolding materials and perfusion bioreactors1-3. However, primary cells are subjected to donor variability and are not suitable to model pathophysiological hematopoiesis in highly reproducible settings. Our goal is to engineer 3D standardized HSPC niches by replacing primary cells by human induced pluripotent stem cells (hiPSCs).

Methodology
Prior to engineer a 3D standardized vascularized osteoblastic niche, we first assessed in static conditions if the WTC hiPSC line4 could differentiate into osteoblasts, perivascular cells and endothelial cells. We cultured these hiPSCs to form aggregates and induced a mesoderm and vascular differentiation for 5 days. These aggregates were embedded into collagen I-Matrigel, and cultured for 11 days more in three different conditions: vascular medium, osteogenic medium and mix of both (1:1). At the end, aggregates were dissociated to analyze their nature by flow cytometry and qPCR. To generate the standardized hematopoietic compartment, we used CD34+ HSPCs that had been differentiated from hiPSCs during 21 days6. The potential of these standardized HSPCs was then assessed by culturing them for 1 and 3 weeks in 3D niches engineered with primary BM mesenchymal stromal cells within perfusion bioreactors1-3. At the end of the culture, tissues were harvested and processed for histological analyses and flow cytometry.

Results
For the stromal compartment, the 1:1 mix of vascular and osteogenic medium allowed the formation of 3D aggregates with CD31+ endothelial cells, CD146+ perivascular cells and CD31- CD146- mesenchymal cells. Gene expression analysis confirmed the expression of vascular-related genes, but we did not observe osteoblastic gene expression, suggesting the lack of osteoblastic differentiation.
For the hematopoietic compartment, a fraction of hiPSC-derived HSPCs were still detected in 3D niches at the end of the culture by flow cytometry, while most of them differentiated into monocytes/macrophages, granulocytes, megakaryocytes, mast cells and erythroid cells. This cellular diversity was confirmed at histological level through hematoxylin-eosin staining and immunofluorescence stainings.

Conclusions
Although 3D structures with vascular cells can be generated with the WTC hiPSC line, the differentiation protocol requires optimization to achieve the osteoblastic differentiation. In contrast, hiPSC-derived HSPCs could be successfully maintained and differentiated into different lineages in 3D engineered niches. Our future research aims to combine the standardized stroma with standardized hematopoietic cells to generate a fully standardized hematopoietic niche model that might be exploited to study human hematopoiesis and/or as drug testing platform.

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keywords: Human hematopoiesis, standardized bone marrow niches, IPSCs, engineering, 3D culture

PS21
Biophysical Therapies - External energy to push internal regeneration

83767278968

TOWARDS NON-INVASIVE DEEP BRAIN STIMULATION THERAPIES FOR NEURODEGENERATIVE DISORDERS

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Alzheimer disease (AD) is the most common cause of dementia and constitutes a major socioeconomic burden. As the underlying cause of AD remains unknown, the clinical need for disease modifying therapies is ever more pressing. Due to the low turnover of neural tissues and the extensive neurodegeneration associated with AD, regenerative medicine therapies are a promising strategy for the treatment of these patients [1]. Enhanced endogenous neurogenesis has been associated with a slower cognitive decline in humans and animal models [4]. Similarly, the delivery of exogenous stem cells has been shown to ameliorate the symptoms and lead to functional recovery [1]. However, limitations related to low cell survival and high immunogenicity significantly hinder clinical efficacy.
Electrical stimulation (ES) has been shown to modulate the proliferation and differentiation of neural stem cells (NSC) and thus, it holds high therapeutic potential [2]. Deep brain stimulation (DBS) is a well-established, FDA-approved approach that has been widely used for the treatment of neurodegenerative disorders. Although pre-clinical evidence has shown that DBS leads to slower cognitive decline in AD, the invasiveness of the approach precludes translation [5].
In this regard, DBS via temporally interfering (TI) electric fields has emerged as a promising strategy for non-invasive ES of neurons at depth. However, its influence on the proliferation and differentiation of NSCs and the effect of different stimulation paradigms remains unexplored.

A high-throughput device for in vitro ES based on TI was developed to study the influence of DBS on NSC fate. The effect of biomimetic ES paradigms on NSC proliferation and differentiation was investigated. Changes in the expression of stem-associated and lineage-specific biomarkers were evaluated using immunofluorescent staining. A stimulation frequency of 10 Hz, mimicking the activity in the developing brain, was proven to increase neuronal differentiation, proliferation, and metabolic activity of NSCs. Transcriptome analysis using mRNA-seq was carried out to investigate changes in gene expression following ES at this frequency. Spontaneous neural network activity in differentiated cultures of NSCs was evaluated using live calcium imaging.
The effect of this stimulation paradigm on the proliferation and differentiation of NSCs encapsulated in an injectable scaffold was also investigated. It has been hypothesised that the combinatorial effect of ES and endogenous microenvironmental cues can improve the survival and integration of exogenous stem cells. Therefore, NSCs were encapsulated in a bioactive, self-assembling hydrogel and stimulated in vitro with the optimised paradigm. The combinatorial effect of ES and the bioactive scaffolds on the maturation and functionality of the resulting neural networks was evaluated as described above.

An ES paradigm for the modulation of NSC fate was optimised in vitro using a bespoke high-throughput stimulation device, and an optimal frequency to enhance neuronal differentiation has been identified. A combinatorial strategy based on NSCs encapsulated in a self-assembling injectable scaffold for minimally invasive delivery and stimulation was also developed. This approach could constitute the basis for non-invasive DBS strategies for the treatment of AD and other neurodegenerative disorders, which can promote endogenous neuroregeneration or increase the efficacy of exogenous stem cell transplants.

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keywords: neuroregeneration, deep brain stimulation, dementia, stem cell therapies

PS22
Bringing together state-of-the-art quantitative biology and machine learning-based modeling for controlling and predicting cell and cell population phenotype in the context of regenerative medicine

62825447766

DATA INTEGRATION IMPORTANCE FOR ENABLING REGION-FREE IMAGE-BASED CELL QUALITY CONTROL

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[Introduction] Image-based analysis of cells is a powerful modality to measure and record the high content information which reflect the cellular status during their culture. By the recent advances of image processing and machine learning technology lead by artificial intelligence (AI) applications in other industrial fields, “image data” will be a new frontier of biological big data which will support the cell-related life sciences. However, cell images around the world have not been standardized among all the cell-handing facilities, since microscopic observation skills and techniques have been the most trusted method to control cell culture process for more than 100 years. Such situation is greatly different from the other industrialized fields, which challenged to abandon the experts’ feeling-based manufacturing manner to evolve to the data-driven manufacturing. To introduce the most advanced technologies for mechanization and automation of cell culture processes to maximize the efficiency and stability of cell-related techniques, we have to prepare for the next stage after the simple introduction of novel technologies in cell culture, the period of “data integration for data-driven activities”. Our group has been reporting the label-free morphology-based analysis approaches for developing enabling technology for cell quality monitoring and control for maximizing the efficiency and reproducibility of cellular researches and manufacturing [1-3]. However, there are still rare researches that report the possibilities and effectiveness of studying the “data integration” of collected “cell images”. Therefore, in this work, we developed the data integration technology for enhancing the future coming “image-based cell quality control process” in cell manufacturing industry.

[Methods] We examined the effect of data bias effect between image differences and facility differences within the same culture process for manufacturing mesenchymal stem cells for therapeutic use. Especially, we examined the image resolution effects, and developed effective image interpolation method for standardizing the morphological descriptor effects for predicting cell yield of mesenchymal stem cells.

[Results and conclusions] From our data, we found that the bias of lighting effect is one of the most crucial noises that disturbs the morphology-based machine learning model performances. Moreover, we also found that the resolution differences between cell culturing facilities can be interpolated effectively to achieve equivalent cell yield prediction performances. This investigation will suggest the importance of our present situation which neglect the image data quality in the data accumulation process for further AI applications. Our data also indicates that morphology-based cell image analysis has higher potency of obtaining robust prediction models compared to the deep network models, since it enables detailed data normalization.

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keywords: mesenchymal stem cells, quality control, data integration

94238132444

LABEL-FREE IMAGE-BASED CELLULAR RESPONSE EVALUATION TECHNOLOGY FOR SUSPENSION-TYPE CELLS

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Suspension-type cells, which do not require scaffold adhesion during their culture, is commonly regarded as cells derived from blood cells. In bio-pharmaceutical researches, antibody-production cells are also another major cell known as suspension-type cells. However, by the recent tissue engineering advancements, more and more types of suspension-type cells are required for cell therapy. Chimeric antigen receptor (CAR) T-cells are one of the cutting-edge cellular products that are expanding worldwide and therefore expected to be manufactured in high quality as medicinal products. Furthermore, suspension-type culture is another important cell culture technique to efficiently scale up the cells for therapy, therefore various types of cells are trying to be cultured in a suspension manner (including the usage of microcarriers or special density medium). However, compared to the adhesion-type cells, their daily quality monitoring had been difficult by conventional microscopies, since suspension-type cells float and move even during the imaging process. Therefore, although there had been numerous image-based analysis techniques that reported effective non-invasive analysis performances to evaluate the culturing cells, there are rare cases reported to apply label-free image-based analysis techniques for suspension-type cells. Our group has been investigating the use of microscopic images to non-invasively evaluate, record, and monitor cellular quality during their culture process for evaluating cell profiles or screening for adhesion cells [1,2]. In this study, we investigated the possibility and effectiveness of applying our method for analyzing suspension-type cells.
[Method] By optimizing the microscopy type and imaging condition, we determined a stable protocol to obtain their morphological features in the micro-well plates. Furthermore, we developed the exhaustive focusing algorithm to obtain "sharp contrast" for every cell in the field of view, to obtain robust and reproducible morphological feature parameters. We intentionally prepared suspension-type cells under culture stress (lactic acid, ammonia), and created cell quality variations. Using multiple morphological feature parameters extracted from the bright-field microscopic images, we analyzed the correlation between the stress condition and their morphological profiles using statistical methods and machine learning models.
[Results and conclusions] From our data, we found that the selection and creation of effective morphological feature parameters for suspension-type cells enable to detect of stress-triggered cell growth arrest only from their morphological profiles. Moreover, we applied our image analysis tools for several suspension-type cells to evaluate the robustness of our analysis technique and found that time-course morphological transition information is important not only for the adhesion-type cells but also the suspension-type cells.

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keywords: Suspension-type cells, morphology, image-based evaluation

PS24
Cell-rich constructs for tissue engineering

41883639519

A MULTI-WELL BIOREACTOR FOR CARTILAGE TISSUE ENGINEERING

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Introduction:
Bioreactors are an important tool to study clinical scenarios under more realistic conditions before heading towards preclinical and clinical studies. We previously showed that, using our standard bioreactor (SB), multiaxial load activates transforming growth factor β1 (TGF-β1), and thereby pushes the mesenchymal stromal cells (MSCs) towards chondrogenesis[1]. Such bioreactors offer the unique opportunity to test tissue engineered constructs under kinematic loading conditions. However, only 4 samples can be run at the same time. Therefore, we developed a multi-well bioreactor (MSB) which allows for the simultaneous multi-axial loading of 16 samples at the same time with additional advantages, such as ease of handling. In this study, we compare the MSB to the SB and showcase how it could be used for further studies revolving around higher throughput testing of cell-laden constructs used in the field of tissue engineering.

Methodology:
MSCs were obtained from three donors after acquiring written consent from the patients (Freiburg, EK-326/08) and encapsulated in fibrin–poly(ester-urethane) scaffolds (5 x 106 cells per scaffold). Samples were cultured in TGF-β1-free chondropermissive medium either in an unloaded state (UL) or subjected to joint-mimicking multiaxial load (combination of shear and compression) within two different bioreactors: a previously used standard bioreactor (SB) and a new multi-sample bioreactor (MSB). The loading protocol consisted of 1 h of loading for 20 days during a period of 25 days. Culture medium was collected and replaced every second to third day. ELISAs were performed to quantify active and total produced TGF-β1. Nitrite was detected using the Griess assay and sulphated glycosaminoglycans (sGAG) were quantified using DMMB assay. The different outputs were all normalized to the DNA content (measured with the Hoechst dye) of the respective samples. Additionally, for time points at day 7 and day 25, gene expression was quantified using real time PCR (both standard plates and array cards) and histological sections were stained using Safranin O / Fast Green (SAFO-FG).

Results:
TGF-β1 was activated only within the SB and MSB groups. MSB showed greater sGAG retention within the samples loaded compared to both SB and UL samples ($p < .001$). In contrast, for MSB samples, the total sGAG content (medium + sample) was lower than in SB and UL samples ($p < .001$). Gene expression revealed an increase in chondrogenic markers (ACAN, COL2, SOX9/RUNX2 ratio) for both SB and MSB compared to UL. MSB samples also showed more enhanced SAFO-FG staining compared to both SB and UL.

Discussion:
We highlight the use of a novel multi-well bioreactor that performs similarly if not better than a standard bioreactor in terms of mechano-induced chondrogenesis. Furthermore, this multi-well bioreactor offers the unique opportunity to apply kinematic like loading to multiple samples at the same time in a user-friendly manner, which could lead to great advantages for swift testing of TE constructs.

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keywords: Bioreactor, stem cells, growth factors, chondrogenesis, mechanobiology

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A NEW SPECIMEN ASSESSMENT TOOL FOR ENABLING TISSUE ENGINEERING PROTOCOL PROGRESSION: SUCCESSFUL INTEGRATION OF PHASE-BASED X-RAY IMAGING IN AN OESOPHAGEAL IN-VITRO MATURATION PROTOCOL

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Introduction

Patient specific, tissue engineered oesophagi represent a possible solution for conditions which currently lack effective therapeutic options, such as long-gap oesophageal atresia1. Histology, the current gold standard for tissue engineering (TE) protocol design, is destructive and 2D, disregarding volumetric information which are essential for confirming the regeneration of the appropriate organ architecture and subsequently function. X-ray phase contrast computed tomography (PC-CT), can generate novel volumetric density maps of organ architecture in a non-destructive manner2,3.

Methods

Laboratory-based edge-illumination PC-CT2 was integrated in an oesophageal TE protocol, imaging 50 piglet derived specimens. This included specimens of type: native (n=20), as extracted from the piglet, scaffold (n=20), native-derived cell-free constructs1, and tissue

engineered (n=10), recellularised - in-vitro matured cell-seeded scaffolds4. A semi-automated, machine learning-based, image processing pipeline was developed for perfuming specimen characterisation5. This entailed 1) the volumetric visualisation and 2) quantitative, intra- and inter-sample comparison, based on oesophageal physical density and morphology. A new, comprehensive specimen evaluation was made possible by combing 1) and 2).

Results/Discussion
All oesophageal layers, classically visualised in 2D using histology, are assessed in 3D in the native oesophagi, their preservation is confirmed in the scaffold specimens, whilst an evident loss in layer architecture is observed in the cell-repopulated portion of the recellularised samples. One of the developed tools, the “radial density profile”, extracts density variation within PC-CT slices, and allows for assessing quantitatively cell-distribution throughout the volume of a recellularised sample in a non-destructive manner. Further to guiding the progression of the existing TE protocol, the imaging tools and developed pipeline demonstrated to be compatible with scale-up models, from piglet to pig derived oesophagi. PC-CT will play a fundamental role in in-vivo testing stages, as its non-destructive nature will eliminate the need for extracting the engineering construct for assessment, and will enable the evaluation of construct integration with the host.

Conclusions
The comprehensive construct evaluation, delivered by PC-CT in a non-destructive manner can unlock the great potential of the field of TE, ultimately allowing for the successful regeneration of organs with complex architecture and function.

Acknowledgements
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keywords: 3D-Imaging, Laboratory-based phase contrast computed tomography (PC-CT), in-vitro organ maturation

20941825929

ASSESSING JELLYFISH COLLAGEN HYDROGEL FOR SUPPORTING FOR HUMAN OSTEOBLASTS

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Introduction:
Large bone defects can be caused by trauma, disease, surgery or tumour resection where the bone is unable to heal as normal due to the size of the defect or fracture [1-3]. To understand and resolve this clinical problem, regenerative medicine approaches, particularly tissue engineering and the use of biomaterial, have been widely studied and used. A more recent type of collagen that has emerged is jellyfish collagen (Jellagen), which is collagen type 0, non-cytotoxic and biocompatible. [4] In this project, we are investigating whether a jellyfish collagen hydrogel can provide a natural 3D microenvironment for bone formation.

Materials and Methodology:
Established quantitative and qualitative methods such as PrestoBlue™ Cell Viability Reagent, Live/Dead ® Viability/Cytotoxicity Kit, DNA quantification and histology have been used to determine whether a jellyfish collagen hydrogel supports human osteoblast viability, proliferation and migration.

Results and Conclusion:
The overall PrestoBlue results showed that the hydrogel supported cell viability and to further support this, the Live/Dead images were strong evidence to show that osteoblasts were viable and proliferating both within and on top of the hydrogel. Moreover, it was evident that osteoblasts seeded within the hydrogel were distributed throughout the hydrogel across the 7 day period. This is a promising result that suggests the cells can and will be able to proliferate long-term in a 3D microenvironment.
The early experiments of this project have so far shown that a jellyfish collagen hydrogel can support human osteoblasts and encourage their growth over 7 days. Some results have shown that cells are viable within the hydrogel at 21 days. This is a good indication that cells can be cultured for longer. The next step would be to assess bone formation in the hydrogel up to 28 days.

Acknowledgements:
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keywords: collagen, hydrogel, microenvironment, viability, osteoblasts

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COAXIAL BIOPRINTING OF CELL-LADEN CORE FILAMENTS USING A HYALURONIC ACID-TYRAMINE BIOINK

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Introduction

Optic nerve sheath meningiomas are the second common optic nerve tumor that affect the optic nerve sheath of primarily middle-aged individuals. In general, invasive measures are avoided, as in most situations, surgery yields no good outcome and is often only considered for blind individuals or those with severe proptosis. Regeneration of the optic nerve fibres bearing newly functional sheaths with the aid of tissue engineering to replace the worn out nerve fibres, could be a long-term solution. Therefore, to regenerate these nerve fibres, it is important to first produce essential tools such as the cell-laden core filaments in vitro. The current research was carried out to produce cell-laden core filaments by coaxially bioprinting a hyaluronic acid-tyramine (HA-TA) bioink using enzymatic crosslinking.

Methods

In this study, bovine primary chondrocytes were isolated and cultured. The confluent cell pellets were combined with tyramine substituted hyaluronic acid of high molecular weight (HMW HA-TA) to form the bioink. This cell-laden bioink was extruded through a coaxial nozzle of 22/16G in a core/shell printing set-up to print the cell-laden core filaments. To improve the gelation mechanism used during the coaxial bioprinting process, biocompatible near-instantaneous enzymatic crosslinking, based on the enzyme horseradish peroxidase (HRP), was explored to yield cell-laden core filaments. The extruded cell-laden core filaments were cultured for up to 7 days in vitro. In addition, the physical properties such as rheological properties, swelling ratio and degradation of the biomaterial (HMW HA-TA) and corresponding hydrogels were examined.

Results

HMW HA-TA was synthesized successfully through DMTMM-mediated amidation. HA-TA with 3 different tyramine substitution degrees (DS) were prepared and analysed. The functional biomaterial was assessed and proven through the formation of gels upon HRP-mediated crosslinking of the phenolic moieties. The gelation time for the enzymatic crosslinking of HMW HA-TA at 3 different percentages (1.3, 1.8 or 2.2 %w/v) with 5.5 U/ml HRP was instantaneous. These cell encapsulated core filaments were printed in the presence of a sacrificial ink sheet based on Pluronic F127. The physical properties of the resulting hydrogel were analysed; the swelling ratio decreased with increasing DS and increased with increasing polymer concentration. The enzymatic degradation rate of the HA hydrogel was inversely proportional to the concentration of polymer and DS.

Conclusion

We developed a near-instantaneous crosslinking HMW HA-TA bioink for the production of cell-laden filaments by core/shell bioprinting, which can be used for nerve fibres' regeneration in the future.

keywords: Coaxial bioprinting, bioink, hyaluronic acid, enzymatic crosslinking

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DECORIN IMPROVES PANCREATIC B-CELL FUNCTION AND REGULATES ECM EXPRESSION IN VITRO

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Pancreatic islet transplantation is a promising therapeutic advancement in the treatment of type 1 diabetes; however, a major obstacle remains in supporting cell function post-transplant. To identify possible extracellular matrix (ECM) proteins for the functionalization of islet encapsulation devices, we investigated the presence of ECM in native pancreatic tissues and in our EndoC-βH3 pseudo-islet system. We identified the small, leucine-rich proteoglycan decorin to be strongly co-localized with insulin-producing cells. Supplementation of decorin in the pseudo-islet cultures significantly increased the β-cell glucose response while also downregulating the ECM proteins involved in fibrosis. Interestingly, these changes in ECM, as well as changes in phospholipids within the endoplasmic reticulum (ER), were also observed through Raman microspectroscopy. Next-generation sequencing of pseudo-islets supplemented with decorin showed upregulation of genes involved in glycolysis and protein transportation within the ER. Furthermore, functionalization of a clinically approved collagen type 1 gel with decorin significantly increased insulin secretion of embedded pseudo-islets in response to glucose. Immunofluorescence staining and Raman microspectroscopy confirmed that decorin impacts ECM remodeling of pseudo-islets within the functionalized gel. Raman microspectroscopy also showed decorin influences the phospholipid content within the ER, suggesting changes in protein trafficking upon decorin supplementation. Our data proposes decorin as a therapeutic agent that supports β-cell function and improves the efficacy of pancreatic islet transplantation.

keywords: decorin, β-cells, fibrosis, glucose-responsiveness

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DESIGN AND OPTIMISATION OF PERFUSION BIOREACTORS FOR LARGE-SCALE MANUFACTURE OF RED BLOOD CELLS

Chan Lee (University of Bath, Bath, United Kingdom)

Tissue engineering has shown and still shows the potential to improve quality of life, especially in the cell therapy industry. An area of interest is in the in vitro production of red blood cells (RBCs). Blood transfusion is one of the most widespread forms of cell-based therapy and has been in use for over 50 years. However, the World Health Organisation (WHO) aims for each country to have 10 units of donated blood for every 1000 people, but studies show more than 40 countries fail to meet the WHO's standard (Weimer et al., 2019). As the supply of blood is not meeting the demand, another sustainable method may be required to subsidise the blood bank, such as in vitro production using bioreactors. The benefit of cultured red blood cells (cRBCs) is the low risk of alloimmunisation, guaranteed availability and disease-free blood due to the sterile production.

Bioreactors have been increasing in popularity within biotechnology, for instance in the production of antibodies, cells, and tissues for therapy, due to their ability to control environmental factors and nutrient levels, enabling optimisation of cell growth and cost reductions. This project will study and improve large-scale manufacture of cells, improving the cell therapy industry and addressing the shortage of donated blood.

The aim of this research is to design and optimize a bench-scale perfusion bioreactor to produce red blood cells (RBCs) from precursor cells. Synthesis of RBCs requires 2 stages: (1) proliferation of the precursor cells to achieve sufficient starting cell numbers, and (2) differentiation, where the precursor cells mature into a specialised RBC. The approach of the project is to design a bench-scale fluidised bed bioreactor (FBB) and focus on the different parameters and their effects on the proliferation and the differentiation of the cultured cells. Next, the scale-up of bioreactors will be studied, to establish the optimal design and operation for large-scale manufacturing of RBCs for clinical use. The results from the above will show how biological properties of the cell are affected by growth within the FBBs. Hydrodynamic data of the system such as minimum fluidisation velocity and pressure drop will be obtained and research on how these affect biological systems will be researched. These data will give the optimum conditions to operate the bioreactor for maximum growth and production of cRBCs.

The bioreactor that will be developed during this project will be a platform technology applicable for manufacturing other similar cell therapies. The findings for this project will be important and applicable for biochemical engineering and tissue engineering, which may improve the understanding of replication and differentiation of cells in bioreactors.

keywords: Bioreactors, Red blood cells, optimisation

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DEVELOPMENT OF BIOADHESIVE MICROCAPSULES AS A NEW CELLULAR TREATMENT FOR THE DIFFUSE CARTILAGE LESIONS

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Introduction
Cartilage structures can be damaged causing two types of injuries: focal; the lesion is restricted to a concrete zone or diffuse; the lesion affects a broad area of the articular cartilage. This type of lesions appears due to the reduced self-healing capacity of articular cartilage. Diffuse damage is much more difficult to treat. As the damage progresses up to the point where the majority of the cartilage is lost, the only option available is the surgical replacement of the arthritic joint with a prosthesis. More recently, cell-based approaches using autologous chondrocytes or mesenchymal stem cells (MSCs) have been tested and it can even be found in some clinics. However, the efficacy of such cell-based treatment is controversial where the main problem is the unconfinement of the cells used. The most promising approach is the combination of such cells with a biomaterial-based carrier, being the general objective of the project the development of bioadhesive and injectable cell microcarriers with the ability to regenerate the articular cartilage. This aim is materialized in the fabrication of multibiofunctional capsules that are able to promote the cell cargo with selective adhesion and location on the articular surface.
The microcapsules are based on a novel kind of biomaterials, named Recombinamers that are polypeptide materials obtained by recombinant DNA technology. In particular, the core composition of the microcarrier is the Elastin-like Recombinamers (ELRs).

Methodology
Recombinant DNA techniques have proven to be very powerful tools for the development of novel protein-based biomaterials. This class includes ELRs, which are protein-based polypeptides that comprise repetitive units of the Val−Pro−Gly−X−Gly (VPGXG)n pentapeptide, in which X (guest residue) could be any amino acid except L-proline. ELRs are inspired by elastin, showing excellent biocompatibility, and they exhibit thermo-responsiveness in aqueous media.
Gene construction were performed using standard genetic-engineering methods. Production was carried out by recombinant techniques using Escherichia coli as the cell system. Purification was performed by several cooling and heating purification cycles (Inverse Transition Cycling) following centrifugation.
The purity and molecular weight were verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Amino acid composition analysis and infrared spectroscopy were also performed.

Results
Genetic engineering methods allowed the synthesis of the genetic construct capable of synthesizing the desired biomaterial. Biopolymer adhesion to the hyaline-cartilage matrix was demonstrated measuring the adhesion forces between our biomaterial and surfaces with collagen II and chondroitin-sulfate versus control surfaces.
Tailored layer-by-layer (LbL) approaches allowed the encapsulation of cell spheroids of

autologous chondrocytes and MSCs.
In vitro and ex vivo assays were performed using cartilage explants from patients surgically intervened, this type of analysis showed the derivatized ELR adhesion efficacy to the articular surface and the final liberation of cell spheroids hoping to demonstrate the cartilage regeneration.

Conclusion
In conclusion, a novel protein-based biomaterial with adhesion to the hyaline-cartilage matrix was synthetized. Besides, cell spheroids were encapsulated by the polymer.
Hence, in this study could be possible overcome the limitations of the current treatments used in diffuse cartilage lesions and it supposes an advance in regenerative medicine.

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keywords: Articular cartilage, biomaterial, elastin-like recombinamers, diffuse lesion

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EFFICIENT HUMAN MUSCLE ENGINEERING RELIES ON THE CORRECT DECELLULARIZATION METHOD

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Introduction
The employment of recellularized autologous extracellular matrices (ECM) as scaffold for the reconstruction of complex tissues like skeletal muscle has been recognized as a successful strategy for regenerative medicine. Compared to the application of synthetic patches, which represent the current standard of care for congenital neonatal malformations like omphalocele and gastroschisis, the implantation of a naturally derived bioengineered construct has great advantages such as providing biochemical and mechanical stimuli driving cell growth and differentiation. However, the decellularization method chosen to produce the scaffold deeply influences its properties and the recellularization efficiency. Therefore, the aim of this work was to study the best method to decellularize human muscle and to prove the recellularization efficiency.

Metodology
Two detergents (sodium deoxycholate and sodium dodecyl sulphate) and latrunculin B were used in cycles of decellularization together with DNase I. At the end of each cycle, the samples were analyzed for residual DNA content, ECM component preservation (collagen network, laminin, glycosaminoglycans), and mechanical properties. Finally, in the matrices produced, human muscle precursor cells were injected to evaluate the recellularization efficiency.

Results
The usage of detergents allowed the obtainment of an 85% reduction in DNA content, but muscle ECM architecture and composition was more impaired with detergents with respect to latruculin B. In addition, a higher amount of residual sulphated glycosaminoglycans was found after treatment with latrunculin B, while the detergents clearly compromised the glycocalyx. Furthermore, in the ECM treated with detergents, cells accumulated only at the border of the samples, while in latrunculin B-treated matrices cells were more evenly distributed, with high proliferative and myogenic capacity.

Conclusions
These results indicate the latrunculin B protocol as the best method for the obtainment of a human skeletal muscle ECM compared with the usage of detergents. The efficient decellularization did not impair native tissue architecture, mechanical properties, and

composition. The high recellularization yield achieved makes it a tool for the in vitro production of a bioengineered skeletal muscle construct, with the final goal to produce implantable tissues for regenerative medicine purposes.

keywords: extracellular matrix, skeletal muscle, decellularization

83767217927

FABRICATION OF POLYSACCHARIDE BASED HYDROGEL VIA ENZYMATIC REACTION FOR CARTILAGE TISSUE ENGINEERING

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Polysaccharide-based hydrogels have been developed in the fabrication of multifunctional microenvironments for tissue fabrication. In this study, two opposite charged polysaccharides including chitosan and hyaluronic acid conjugated with phenol moieties (PH) (CH-PH &HA-PH respectively were mixed at different ratios and utilized to enzymatic cross-linking in the presence of H2O2 as an electron donor to make hybrid hydrogel scaffolds for cartilage tissue engineering. The physical characteristics of hydrogels including gelation time and water contact angle were measured and results showed decreased these properties with increasing HA-PH content. Meanwhile, the compressive modulus and strength of the hydrogels increased with the amount of HA-PH. The cellular studies showed proper cell viability and proliferation for cells on optimum hydrogel surface (CHA-PH2) compared with neat hydrogels. The MSCs were able to undergo robust chondrogenesis and generate the proper cartilage scaffolds in hybrid CH-PH with optimum concentration of HA-PH named CHA-PH2 indicated by the real-time polymerase chain reaction of Col2, SOX9, and ACAN, and mechanical testing suggesting its potential in future in vivo model for the repair of cartilage defects. In summary, the results demonstrated the applicability of developed polysaccharide-based hydrogel in cartilage tissue engineering.

keywords: Chitosan, Hyaluronic acid, Horseradish peroxidase reaction, Cellular growth, Cartilage tissue engineering.

20941816086

GELMA HYDROGELS: TOWARDS THE DEVELOPMENT OF AN IN VITRO 3D MODEL OF THE HUMAN ENDOMETRIUM

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INTRODUCTION
The endometrium is a complex, multicellular tissue and is the site of embryo implantation. Dysregulations between embryo-endometrial interactions during implantation result in a variety of pregnancy disorders including recurrent pregnancy loss and implantation failure. Due to a lack of appropriate study models the mechanisms surrounding human embryo implantation are still largely unknown. Here, we describe how photocrosslinked GelMA hydrogels can be tailored to the culture of both endometrial stromal cells (ESCs) and endometrial organoids (EOs), which have been shown to recapitulate key features of in vivo endometrial glands.2,3 Our long-term goal is to engineer a platform to assess the processes that control implantation and address unanswered questions around reproductive failure.

METHODOLOGY
Two batches of GelMA with a degree of substitution (DS) of 70 & 100 % were prepared.1 5 and 10 % (w/v) GelMA hydrogels were fabricated, and their mechanical properties measured using rheology.
A simple protocol was developed whereby primary human endometrial cells were encapsulated in photocrosslinked GelMA hydrogels, employing a cytocompatible photoinitiator (LAP) and 3 minutes UV exposure (365 nm).
ESC viability in GelMA hydrogels was investigated using the XTT Cell Viability assay. The ability of ESCs to respond to steroid hormone treatment was examined using a prolactin ELISA. Collagen was used as a control.
Time-lapse imaging was used to observe the ability of epithelial cells encapsulated in GelMA hydrogels to form EOs. GelMA hydrogels were supplemented with the laminin protein at 2 different ratios. Geltrex was used as a control.

RESULTS
Rheological analysis demonstrates GelMA hydrogels can be produced with a range of stiffness degrees by altering the DS and concentration of GelMA.
ESCs in DS70 and DS100 GelMA hydrogels retained high levels of viability after a 7-day culture period, surpassing the commercially available collagen hydrogel. A clear differentiation response, monitored over 4-days of hormone treatment, was seen in both GelMA and collagen hydrogels.
Endometrial glandular organoids form in the presence of an appropriate 3D matrix. DS70 GelMA hydrogels resulted in a matrix too soft to support organoid formation. DS100 GelMA hydrogels provide a stiffer matrix, which enabled epithelial cells to form EOs. The efficiency of organoid formation could be enhanced through addition of the basement membrane protein, laminin to GelMA hydrogels.
Although optimal hydrogel compositions need to be determined for the co-culture of stromal and epithelial organoids, the results of this work suggest that GelMA hydrogels constitute versatile and tunable hydrogels for endometrial tissue modelling.

CONCLUSIONS
The mechanical properties of GelMA hydrogels can be tuned to suit the culture of different cell types.
Endometrial cells can quickly and simply be encapsulated in photocrosslinked GelMA hydrogels and maintain high cell viability.
Epithelial cells grown in DS100 GelMA supplemented with laminin form structures that resemble in vivo endometrial glands.

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keywords: GELMA,ENDOMETRIUM,IN-VITRO MODEL

20941815426

GENERATION AND CHARACTERIZATION OF A BIOARTIFICIAL COMMON BILE DUCT

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Cholangiopathies (CPs) are a set of diseases affecting the biliary tree and are a significant cause of mortality in adults and children1. Some CPs including biliary atresia, distal cholangiocarcinoma and iatrogenic injures affect in particular the common bile duct (CBD) with strictures leading to liver failure. Therapeutic options for these disorders are limited and rely mainly on the anastomosis of the CBD to the jejunum, which is associated with duodenal ulcers, retrograde infections, and stenosis. Liver transplantation remains the most effective therapeutic option2, but due to the shortage of donor organ and associated costs1, new therapeutic alternatives are needed. The bioengineering of bile ducts represents a promising strategy to restore bile flow continuity2 for CBD disorders. However, the results of this research have been often inconclusive and poorly translatable. Our research group is working on optimizing both the cellular source and the biomaterials, which represent the main challenges of this research field, with the prospect of creating an implantable bioartificial CBD (BCBD). For its fabrication two techniques are used. First, cholangiocytes are mixed with photocollagen at 3 million cells/mL of polymer. Then, the cell-embedded photocollagen is poured into a plastic mold and incubated at 37°C for 30 minutes. The mold is therefore opened and the hydrogel is photopolymerized by UV exposure for complete crosslinking. An external coating is added by dipping the cell-embedded hydrogel pipe into an agarose solution. The BCBD is therefore transferred into the culture medium. Alternatively, electrospun polyurethane-fibers replace agarose on the outer surface of the photocollagen tube. Optimization of cell culture parameters, including photocollagen concentration, cell density, and length of culture, are conducted in small-scale experiments in 96-multiwell plates using disks of cell-embedded material. Mechanical (uniaxial and circumferential tensile testing, dynamic compliance) and cell characterization (cell viability, functionality, scaffold colonization) are performed to select the best parameters for obtaining a functional BCBD. We obtained a BCBD of dimensions similar to the native CBD: 60mm long, 1mm thick, and inner diameter of 5.5mm. For preliminary characterization, we used HuCCT1 intrahepatic cholangiocarcinoma cells, which are embedded in disks of 4-8mg/mL photocollagen and at 1-5 million cells/mL photocollagen. We demonstrate that cells embedded in 6mg/mL photocollagen and at 3 million cells/mL show the best viability profile overtime (up to 14 days of culture) and express biliary markers including cytokeratin 7 and cystic fibrosis transmembrane regulator. We obtained a human-sized BCBD through a simple, inexpensive and easy-to-scale method. We optimized some cell parameters and many other characterizations of cells and materials are ongoing. We trust that the our BCBD, consisting on materials commonly used in tissue engineering3-5 and a cell source already proven to be suitable for our purpose6, will be ready for a preclinical assessment soon.

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keywords: Cholangiopathies, tissue engineering, 3D-cell culture, bioartificial organs, electrospinning

52354533405

HYBRID ELECTROSPUN NANOFIBERS SCAFFOLD COMBINED WITH HUMAN DENTAL PULP STEM CELLS FOR TISSUE ENGINEERING

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Introduction: Tissue engineering approaches appear highly promising for the regeneration of injured bone tissues. This strategy combine three essential components such as: scaffolds, mesenchymal stem cells (MSCs) and growth factors and is based on the culture of stem or progenitor cells on scaffolds in order to generate new bone by osteoinductive cue. Recently bioengineering has been focused on electrospinning technique use to fabricate a nanofibrous mat with appropriate pore size and internal/external scaffold geometry suitable for stem cells growth. Natural and synthetic polymers electrospun scaffolds with seeded stem cells have drawn great interest in tissue engineering. The aim of this study was to investigate the impact of an hydrolytically modified hybrid electrospun nonwoven poly(-L-lactide) and co-caprolactone (PLCL) scaffold on biological behaviour of human dental pulp stem cells (hDPSCs) growing on the nanofiber scaffold.

Methodology: Hybrid hydrolytically modified nanofibrous scaffold was fabricated by blending different weight ratio of poly(-L-lactide) and co-caprolactone. Stem cells were isolated from human dental pulp of extracted the third molars taken from four healthy donors. hDPSCs were seeded on PLCL for evaluation of cell viability, adhesion, proliferation, migration, and osteogenic differentiation. After one, three and seven days of hDPSCs seeding on PLCL scaffold the viability and proliferation of hDPSC was measured using the MTT assay and fluorescence intensity after cells staining with PKH26. hDPSC adhesion to PLCL scaffold was determined through cells counting, and migration was assessed through the hematoxylin-eosin staining of hDPSCs-PLCL samples. hDPSCs osteogenic potential was confirmed by mineralization status detected by Alizarin Red staining and bone-related gene expression evaluated by qRT-PCR analysis. As a control for all experiments, the hDPSCs were cultured as a monolayer.

Results: The results showed that the PLCL scaffold supported hDPSC viability and proliferation. The hDPSCs adhesion rate on PLCL increased with time of culture and was significantly higher compared to control group ($p < 0.001$). hDPSCs were able to migrate inside the PLCL electrospun

scaffold after 7 days of seeding. No differences in morphology and immunophenotype of hDPSCs grown on PLCL and in flasks was observed. The mRNA levels of bone-related genes (OCN, OPN, BSP, DSPP) were significantly higher in hDPSCs after osteogenic differentiation on PLCL compared with undifferentiated hDPSCs on PLCL ($p < 0.005$). The ability of osteogenic differentiation of hDPSCs was also confirmed by the presence of mineral deposits on PLCL by Alizarin Red staining.

Conclusions: This study confirmed that the mechanical properties of a modified PLCL mat support hDPSC attachment and viability/proliferation. The biological features such as: adhesion, proliferation and migration of hDPSCs growing on PLCL scaffold, and good PLCL biocompatibility with stem cells indicate that this mat may be applied in designing of a bioactive hDPSCs-PLCL construct for tissue engineering. Moreover, high osteogenic potential of hDPSCs growing on PLCL suggest that this mat provide appropriate environment for dental stem cells differentiation into osteoblasts and might be used in bone tissue engineering.

keywords: electrospun scaffold; biocompatibility; human dental pulp stem cells; tissue engineering

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LESSONS LEARNED FORM ADIPOSE TISSUE ENGINEERING FOR APPLICATIONS IN BIOMEDICINE AND CULTURED MEAT

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Adipose tissue plays a crucial role in the human body including heat regulation, energy and hormone homeostasis. The clinical need of adipose tissue is mainly found in reconstructive and plastic surgery, e.g. after tumor resection or to treat deep wounds. The aim of tissue engineering is to build-up vital and functional 3D-tissue models in vitro. Furthermode, adipose tissue is an important part of cultured meat in food science. For both applications it is important to optimize the culture condition of primary adipose dericed stem cells (ASCs) and mature adipoctes (ACs).
In the last years we optimized numerous aspects for gaining a reproducible and physiological adipose cell and tissue culture. In detail we developed defined media for culture, differentiation and long term culture, analyzed different biomaterials for 3D-setup and evaluated usability as bioinks in bioprinting. Further, we validated different coculture setups e.g. in combination with endothelial or immune cells. We successfully generated spheroid cultures with ASCs. Moreover 3D tissue models were build-up manually or by using bioprinting techniques.
Viability, differentation and function of the ASCs and ACs were evaluated by different stainings, ELISAs, quantitative assays (leptin, glycerol and western blots after severeal days and weeks of culture. In addition, we tested different biopolymers as scaffold material for 3D-adipose tissue models such as gelatin, collagen, gellan gum etc.. In other studies we used in vitro cell derived ECM produced by ASCs for getting improved culture conditions.
In summary, we have developed optimized culture and differentation protocols (like setup of defined media) for 2D and 3D culture ASCs and ACs as well as cocultures with other cells. For bioprinting of the 3D-models tailor-made bioinks were developed. We were able to generate different setups of functional 3D-adipose tissue models and maintained their functionality over several weeks.

keywords: Adipose Tissue Engineering, adipose derived stem cells, adipocytes, 3D-tissue models, coculture, bioprinting, spheroids

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MOVING FROM 2D INTO 3D: BIOMIMETIC IMPLICATIONS OF MECHANOTRANSDUCER COMPLEX YAP/TAZ IN HUMAN NEURAL AND MESENCHYMAL STEM CELLS

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Introduction: YAP/TAZ complex is considered to be the main player in the mechanotransduction process. It shuttles between cell nuclei and cytoplasm depending on the stiffness of the growth substrate, dimensionality and cell shape. Stem cells in their natural environment, called niche, grow in three dimensional soft tissue but in standard in vitro culture are maintained mostly on flat stiff culture substrates as plastic or glass. These changes of microenvironmental cues have a great impact on the stem cells developmental processes, such as proliferation, differentiation and production of therapeutically active, immunomodulatory factors. Thus to improve and direct the pro-regenerative potential of stem cells, it is highly important to identify the molecular mechanisms involved in sensing the niche by stem cells. Methodology: Here we used two different populations of human stem cells regarding their biology and therapeutic outcome: neural stem cells derived from induced pluripotent stem cells (hiPSC-NSC) and mesenchymal stem cells derived from Wharton's Jelly of umbilical cord (WJ-MSC). Using immunocytochemical analysis we have shown the localization of YAP/TAZ in cells grown on 2D substrates and 3D aggregates and/or fibrin and geltrex- based hydrogel scaffolds in the time dependent manner. In addition, using immunoassay test, we have verified the level of released immunomodulatory proteins after exposition of stem cells to the YAP/TAZ complex inhibitor in different microenvironmental conditions. Results: In both tested human stem cell populations grown in 2D conditions we have confirmed mostly nuclear localization of YAP/TAZ. Already 30 minutes after 3D cell aggregates were created from 2D culture, changes in the distribution of YAP/TAZ complex in the nuclear vs cytoplasmic compartment were observed. Moreover, in 2D conditions, exposure of stem cells to verteporfin (YAP/TAZ inhibitor) induced changes in cell morphology and localization of investigated complex to the cytoplasmic compartment, similar to that found in 3D cell aggregates as well as scaffolds based structures. Spatial conditions of the culture and treatment with verteporfin, both influenced the secretion of immunomodulatory factors (TSG-6, TGFβ, IL-11, and COX2) and differentiation of hiPSC-NSC and WJ-MSC. Conclusions: Mechanotransducer complex YAP/TAZ is involved in fast response of the stem cells to the changes in the spatial organization of the microenvironment with implication on the production of factors that may contribute to their regenerative potential.

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keywords: human stem cells, mechanotransducion, hydrogel scaffolds

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NEW RECELLULARIZATED CORNEAL LIMBUS XENOGRAFTS

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Introduction
Tissue decellularization methods allow obtaining new sources of biological scaffolds obtained from natural tissues and organs1,2. Although decellularized scaffolds can be useful, it is well known that their regenerative potential is significantly improved after recellularization. In the present work, we evaluated the potential of several cell sources to recellularize a xenograft scaffold obtained by porcine sclerocorneal limbus decellularization.

Methodology
Porcine corneal limbi were decellularized with 0.1% SDS and thoroughly washed in PBS. Then, decellularized xenografts were recellularized using three different cell sources: cultured corneal epithelial cells (CEC), human adipose derived stem cells (hADSC) and human umbilical cord Wharton's Jelly stem cells (hWJSC). To favor recellularization, xenografts were casted in agarose molds. Cell recellularization efficacy, limbal stem cell maintenance markers and extracellular matrix (ECM) components were evaluated by histochemical and immunohistochemical methods after 7, 14 and 21 days of ex vivo development.

Results
We found that the recellularization efficiency was different for each cell source. First, we found that CEC were able to attach to the decellularized xenografts and formed a partially stratified epithelium able to express several cytokeratins and limbal stem cell markers from day 7. When hADSC were used, we found that cells attached to the xenograft from day 14-21 of development, with expression of some of the limbal markers. However, we found that hWJSC were not able to efficiently attach and grow on the decellularized tissues during the 21 days of the study.

Conclusions
These results suggest that the decellularized xenografts used in this study can be efficiently recellularized with CEC and, to a lesser extent, with hADSC. Expression of several key markers

of limbal stem cells suggest that recellularized cells are able to adapt to the microenvironment of the decellularized tissue kept in culture. These findings suggest that sclerocorneal limbi generated by decellularization-recellularization could have potential clinical usefulness for the treatment of patients with limbal damage or stem cell deficiency.

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keywords: Sclerocorneal limbus, Limbal stem cell deficiency, Decellularisation, Recellularization, Xenograft.

94238150139

PLATFORM TECHNOLOGY TO ENHANCE THE GROWTH OF HUMAN SKIN MODELS IN-VITRO, FOR USE IN BIOMEDICAL RESEARCH AND THE ASSESSMENT OF NEW MOLECULAR ENTITIES

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Introduction
In-vitro models advanced significantly with the introduction of 3D culture technology providing a physiologically relevant microenvironment. While tissue morphology and functionality have greatly improved, bioengineered tissue models often lack long-term stability with a decrease in cell viability and tissue integrity, preventing their use for chronic exposure and long-term studies in wound healing. Traditional 3D methods lack precise control of culture parameters, therefore we have utilised a novel perfusion platform that allows for enhanced control of the in-vitro microenvironment to promote improved replication of native physiological conditions.

Methodology
We aim at improving the longevity of our in-vitro full-thickness skin model by fine-tuning the culture microenvironment, considering the nutrient-metabolite-signalling factor balance, gaseous environmental conditions and simulation of in-vivo perfusion. Skin models are generated by culturing human primary fibroblasts in the porous Alvetex® scaffold, providing an ideal 3D environment for cell infiltration and ECM deposition, and subsequent seeding of primary human keratinocytes onto the robust dermis to form a multi-layered and stratified epidermis at the air-liquid interface. Dynamic culture conditions were introduced using in-house built and commercially available perfusion systems with adjustable flow velocities at different culture stages also allowing for variation of the flow pattern. Using computational fluid dynamics, fluid flow can be characterised to predict shear stress and mass transfer.

Results
Analysis of co-factors during skin model maturation demonstrated their stability under common culture conditions and revealed the superior significance of nutrient-metabolite balance for functional tissue maturation in models generated from fibroblasts obtained from varied age groups. The integration of dynamic culture phases through perfusion enhanced scaffold infiltration and build-up of multiple cell layers. Analysis of extracellular matrix proteins such as Collagen I demonstrated increased deposition and improved distribution uniformity throughout the model. In low-serum media, perfusion was found to shorten the tissue maturation period which allowed for the formation of a robust overlying epidermis at an earlier time point, reducing overall model maturation time. Improved maturation seems to be achieved through advanced cell differentiation mediated by signalling factors such as TGF-β1. This result was found to be transferable across different perfusion platforms investigated in these studies.

Conclusion
Perfusion is a valuable tool to bridge the gap between in-vitro and in-vivo conditions. Understanding how different perfusion patterns affect flow characteristics and in turn gene expression, endogenous protein production and the build-up or disruption of signalling factor gradients, will support the development of long-term skin model cultures with maintained tissue functionality. Ultimately, this will allow for prolonged studies to be carried out to increase the significance of in-vitro research.

keywords: skin, perfusion, in-vitro model

62825432286

PROTEOMIC ANALYSIS OF A HUMAN LYOPHILIZED 3D SCAFFOLD FREE TISSUE ENGINEERED PRODUCT FOR BONE RECONSTRUCTION.

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Introduction
Bone tissue engineering is a promising therapeutic approach for bone reconstruction, which has been successfully applied in clinical trials. NVDX3 is an allogenic and off-the-shelf candidate advanced therapy intended for use as bone void filling materials. It is a lyophilized, terminally sterilized powder derived from a 3-D cell product containing extracellular matrix and osteodifferentiated cells associated with hydroxyapatite/beta-tricalcium phosphate (HA/βTCP) particles. Based on in vitro and in vivo data, we showed that NVDX3 leads to bone formation enabling induction of endochondral ossification and inhibition of osteoclasia.

Methodology
To address the protein composition of NVDX3 product, deep proteomic profiling was performed on samples derived from different stages of the manufacturing process of three independent batches. For the unbiased quantification of all detectable peptides and proteins, HRM™ (Hyper Reaction Monitoring) mass spectrometry was applied on the tested samples. HRM mass spectrometric data were analyzed using Spectronaut software. Corresponding proteomic networks were analyzed via the UniProt Consortium and STRING consortium databases.

Results
More than 5500 proteins were identified in the drug substance (DS) and the final product (DP) of NVDX3, among which 233 were consistently upregulated during the maturation of the 3-D cell product (CP) and maintained in the final product. Untargeted analysis on the most abundant proteins in the product or the most upregulated ones during the manufacturing process showed a strong correlation with collagen-containing extracellular matrix (ECM), blood vessel development and bone growth. To identify potential key players for the activity of the product, a targeted proteomic analysis approach was also performed revealing >170 proteins implicated in extracellular matrix composition, >30 proteins involved in bone development, including markers of endochondral bone formation, and >10 factors with angiogenic or antiresorptive properties.

Conclusion
NVDX3 product composition is characterized by the presence of collagen-containing ECM components which have been produced during the manufacturing process and it includes growth factors known to have an osteogenic, angiogenic and anti-resorptive activity. The deep proteomic analysis allows to identify key attributes involved in the mode of action of the product and to better understand the mechanism of bone formation on tissue explant from in vivo studies.

keywords: stem cells, bone, extracellular matrix, proteomic, tissue engineering

41883650155

TISSUE-ENGINEERED NEURAL TISSUE INTERFACES FOR NEXT GENERATION BIONIC DEVICES

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INTRODUCTION
Bionic implants have been widely used in the clinic to replace or restore impaired neurological function [1]. However, conventional devices rely on stiff metallic electrodes, which often trigger inflammatory responses that hinder longevity and performance [2]. Although different biomaterials have been investigated to dampen the mechanics of stiff electrodes, the establishment of a chronically stable device-tissue interface remains challenging.
It has been hypothesised that tissue-engineered interfaces containing live neural cells could promote the biological integration of implantable devices. Furthermore, the establishment of synaptic communication between bionic implants and the nervous system would enable safer and more natural modes of tissue activation. However, the development of these “living electrodes” has been hindered by the lack of biomaterial-carriers that support the development of the encapsulated cells into functional neural networks [3].
Herein, a biosynthetic hydrogel system based on gelatin (GEL) and norbornene-functionalised poly(vinyl alcohol) (PVA) was developed. The synthetic PVA matrix was essential to tailor the mechanical properties and provide stability to the construct. Conversely, the RGD and MMP-2 sensitive motifs from gelatin provided sites for cell adhesion and enzymatic degradation, respectively. Furthermore, recent studies have shown that glial populations are needed to support neuronal development and function in vitro [4]. Hence, the ability of PVA-GEL hydrogels to support the growth of encapsulated primary astrocytes and co-cultures of primary neural cells was evaluated.

METHODS & RESULTS
Primary rat astrocytes were encapsulated in PVA-GEL hydrogels. Cytoskeletal development and cell-material interactions were evaluated via immunofluorescent staining (IFS) of GFAP and paxillin, as well as MMP-2 production in 3D. These results showed that encapsulated cells were able to adhere and develop focal adhesions, while also engaging actively in matrix remodelling to mediate cell migration. Subsequently, primary rat neural stem cells were encapsulated in combination with mature primary astrocytes. The morphology and functionality of the resulting neural constructs was assessed via IFS and calcium imaging. These results showed that PVA-GEL hydrogels were able to support the formation of functional complex co-culture systems in a 3D environment. In addition, the ability of these neural constructs to interface with neural tissues was evaluated using organotypic cultures. Rat brain slices were cultured on top of cell-laden PVA-GEL hydrogels and the scaffold-tissue interface was analysed using IFS of neuronal, astrocytic, and pre- and post-synaptic markers. The results of these organotypic cultures showed seamless integration between cell-laden hydrogels and rat brain slices and IFS suggested the establishment of functional synaptic connections between the two layers.

CONCLUSION
A biosynthetic hydrogel with tailorable biological and physical properties was developed. The incorporation of gelatin enhanced cell growth and adhesion by providing biological and topological cues to the encapsulated cells. The incorporation of a glial component was essential to mediate neural growth and development and the formation of functional neural

networks. The engineered neural constructs were shown to interface with the brain slices and support tissue ingrowth in vitro. Therefore, this hydrogel system holds great potential for the development of seamless neural interfaces to mediate the communication between implantable bionic devices and physiological tissues.

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keywords: Biosynthetic Hydrogel, Neural Tissue Engineering, Bionic Devices, Neural Interfaces

PS25
Cellular senescence in tissue damage and regeneration

41883632586

A NEW STRATEGY TO MODULATE CHONDROCYTE SENESCENCE AND REDUCE OSTEOARTHRITIS PROGRESSION

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Introduction.
Osteoarthritis (OA) is a highly prevalent musculoskeletal disease characterized by a chronic inflammation and joint disability. OA is characterized by cartilage degradation and bone overgrowth. Current pharmacological approaches have a merely symptomatic effect and do not delay disease progression.
Several studies have pointed out that chondrocyte senesce contribute to the pathogenesis of OA. Senescent cells secrete cytokines and proteases, that produce inflammation, decrease cartilage regeneration, and promote OA progression. Therefore, the development of an OA therapy aimed to decrease senescence could be a promising alternative for OA control.
The objective of this work is to develop a controlled release system designed to reduce the senescent cells present in OA patients' cartilage and to increase their apoptosis to promote a healthy cartilage homeostasis.

Methodology.
Systems development: Polymeric systems loaded with a senolytic drug were prepared and characterized in terms of drug loading (DL) and loading efficiency (LE).
Osteoarthritic chondrocytes isolation: Cartilage samples obtained from patients with OA who underwent total knee replacement surgery were extracted. Samples were washed and digested with 1 mg/mL pronase and with 1 mg/mL collagenase P following the procedure described by Scotece et al1. The resulting chondrocyte pellet was resuspended in culture medium and seeded in 24-well plates.
Assessment of chondrocyte senescence, caspase-3 activity and LDH secretion: Primary chondrocytes were seeded at 62,500 cells/well in 24-well plates. Then cells were stimulated with TNF-α (10 ng/mL) and treated with the senolytic drug or with the drug-loaded delivery systems for 24 hours. Non-stimulated cells were used as negative control. Cell senescence was evaluated by analysing chondrocytes β-galactosidase activity using the senescence cells histochemical staining kit (Sigma Aldrich). Caspase-3 activity was quantified by the caspase 3 Assay kit (Sigma Aldrich) and LDH secretion was assessed by the cytotoxicity detection kit (Roche) following the manufacturer's protocols.

Results.
The polymeric delivery systems were characterized by a high drug LE (110.0 ± 3.6) indicating their suitability to incorporate the therapeutic molecule.

The treatment with TNF- α produced an increase in the number of senescent cells as expected. On the other hand, the samples of stimulated chondrocytes treated with the senolytic drug or with the polymeric systems presented a decrease in the number of senescent cells. These findings indicate an anti-senescence effect of the developed systems.

The caspase-3 activity is highly related to the lactate dehydrogenase (LDH) release. In this sense,

the stimulation with TNF-α also led to an increase in the chondrocytes' caspase-3 activity and LDH release. The treatment with the drug alone or loaded in the systems further increased the caspase-3 activity and the LDH release of the cells compared to unstimulated cells.

Conclusions.
The developed polymeric systems allowed to reduce senescence and to increase the apoptosis of OA chondrocytes. Similar activity profile was found for both, the senolytic agent alone and the developed drug delivery system.

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keywords: Osteoarthritis, Senescence, Apoptosis, Polymeric systems

62825418284

C-MYC PATHWAY MODULATION IN CELL ACTIVATED BY PLATELET LYSATE STIMULATION

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Introduction: Bioactive factors contained in Platelet Lysate (PL) promote the isolation and proliferation of Mesenchymal Stem Cells (MSCs) and the growth of cell lines. It is well known that PL not only enhances cell proliferation compared to Fetal Bovine Serum (FBS) without affecting the cell differentiation capability, but it is also able to activate resting quiescent cells to re-enter cell cycle. The main proliferation-relate pathway, involving Cyclin, Akt and ERK1/2, is activated after a short exposure of the cells to PL but the effect on proliferation is higher in primary cell cultures than in cell lines.
To better understand this effect, we focused our attention on a family of highly conserved proteins involved in different cellular mechanisms: C-MYC's family. This protein has three different isoforms: C-MYC1 particularly expressed in those cells that are in suffering condition; C-MYC2 expressed in growing cells and C-MYCS, a short isoform whose expression is transient during cell growth.

Methodology: MSCs from different tissues, Articular Chondrocytes, Osteoblasts, Amniotic Fluid Stem Cells from human samples and HeLa cell line, were cultivated in 10% FBS or 5% PL-supplemented medium. The proliferation capability was evaluated in terms of cell doublings and Ki67 expression by immunofluorescence and the possible apoptotic effect evaluating Annexin V by FACS analysis and C-MYC protein expression by Western Blot.

Results: All primary cell cultures treated with PL showed a high proliferation rate in comparison with FBS treated cultures. C-MYC1 expression was absent in FBS cultured cells but was observed in presence of PL also after a short time of exposure. This pattern of expression was different for the cell line where C-MYC1 was present also in the FBS-cultured cells. C-MYC2 isoform was expressed in all the cells independently from the culture conditions. Immunofluorescence analysis indicated that MSC cultures showing C-MYC1 protein expression were in a proliferative stage.
We also find that the cells treated with PL was not in an apoptotic stage, showing only a 15% of Annexin V positive cells.

Conclusions: PL induces cell proliferation increasing C-MYC1 isoform expression in primary cell cultures and much more slightly in the cell line, modifying the described apoptotic function of C-MYC1 isoform. These results pave the way to a deeper molecular study in order to understand peculiar differences of PL stimulation on various types of cells.

keywords: PL culture medium, primary cell culture, cell line

73296345099

EFFECTS OF CELLULAR SENESCENCE ON MECHANOSENSATION: IMPLICATIONS FOR TISSUE REGENERATION

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Introduction
Cellular senescence is an irreversible cell-cycle arrest program that has been associated with numerous biological processes. While emerging studies have extended its role to tissue repair, the focus has been on the effect of the senescence-associated secretory phenotype (SASP) rather than the direct role of the program beyond its paracrine effect. Using a previously established in vitro scaffold-based tissue wound healing model[1], our group recently observed how senescence differentialy affects ECM deposition and tissue tension. Differences observed at the cellular level (morphology, adhesion, cellular forces, migration) directly point to an altered mechano-sensation. We hypothesise that the also observed differences in tissue contraction are influenced by an altered mechano-sensation of senescent cells which in turn leads to distinct cellular responses. We here aim to investigate potential changes in cellular mechanotransduction in senescence due to alterations of mechanical stiffness in the cell environment and aim to link these findings to differences in key processes of tissue regeneration.

Methodology
To investigate individual aspects of the program, senescence was induced to primary human dermal fibroblasts (hdFs) through three triggers: (1) serial passaging leading to replicative senescence, (2) induction of DNA damage through Mitomycin C, and (3) inducible over-expression of p16Ink4a cyclin-dependent kinase inhibitor. To validate the senescence phenotype, cell cycle arrest was assessed through proliferation assays and further characterised by immunoblotting of cell-cycle inhibitor markers (p16Ink4a, p21Waf1). Lysosomal mass was quantified through enzymatic staining of senescence-associated beta-galactosidasde (SA-β-gal) and the phosphorylation of the histone H2AX was identified by immunoblotting.
Substrate stiffness-mediated response was assessed using tuneable stiffness-gradient [2] and uniform-stiffness polyacrylamide (PA) gels. Stiffness of these substrates was validated through nanoindentation. Stiffness-mediated morphological alterations as well as localization of mechanosensitive proteins (YAP, LaminA) were investigated using immunofluorescence staining.

Results
The senescence phenotype was validated by a reduced proliferation, elevated SA-β-gal activity and over-expression of both cell-cycle inhibitor markers and H2AX.
Adjusting the concentration of acrylamide and bis-acrylamide to 12% and 4%, respectively, lead to the fabrication of PA gels with a linear gradient ranging from 1.5kPa to 45kPa. Preliminary results across the gradient, show alterations of senescent cells in terms of aspect ratio and cell spreading. First observations when comparing uniformly soft (1.7kPa) with uniformly stiff (48.3kPa) environments, further support these stiffness-mediated morphological alterations.
In addition, nuclear expression levels of YAP on stiffer substrates appear to be reduced in senescent cells, indicating potential differences in mechanotransduction induction mechanisms of these cells in response to stiffness.

Conclusion
Overall these results will contribute to a so far unknown understanding about the mechanosensitivity of senescent cells. This can help identify particular mechanical environments favoured by these cells which could be integrated into current strategies for tissue regeneration. We plan to further investigate stiffness-mediated alterations of focal adhesion distribution as well as potential stiffness-dependent differentiation (e.g. myo-fibroblast). As a next step, we will study the consequences of senescence on cell organization and tissue defect healing in the above-mentioned scaffold-based in vitro model.

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keywords: cellular senescence, mechanosensation, substrate stiffness

52354539969

HYPOXIA REVEALS A NEW FUNCTION OF FOXN1 IN THE KERATINOCYTE ANTIOXIDANT DEFENCE SYSTEM

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Introduction
The skin, as the outermost barrier of the body, is exposed to threats from the external environment including injuries, oxidative stress and UV irradiation. Therefore, efficient systems of repair and antioxidant protection of damaged skin that are achieved by an extraordinary mechanism involving the interaction of cells, cytokines and growth factors are vital for survival. In the present study, we focused on keratinocytes, the transcription factor Foxn1 and oxygen availability, the components that collectively sustain skin homeostasis and regulate the response to injury.

Methodology
To investigate a possible mechanism by which Foxn1 under hypoxic or normoxic conditions regulates physiological changes in keratinocytes at the molecular and functional levels, we used detailed mass spectrometry analysis (LC-MS/MS) followed by in vitro and in vivo experiments. Primary cultures of mouse keratinocytes transduced with Ad-Foxn1 or Ad-GFP (control) were cocultured with mouse dermal fibroblasts (DFs) under hypoxia (1% O2) or normoxia (21% O2) for 24 h. Afterwards, keratinocytes were used to detailed analyzes: proteomics (LC-MS/ MS mass spectrometry), flow cytometry, qRT-PCR and colorimetric tests. To confirm the proteomics results at the systemic level, uninjured and injured skin collected from the Foxn1-/- and Foxn1+/+ mice using qRT-PCR, Western blot and immunohistochemistry were analyzed.

Results
We demonstrated that Foxn1 in keratinocytes regulates elements of the electron transport chain and participates in thioredoxin system (Txn2, Txnrd3, Srxn1) induction, particularly in a hypoxic environment. Remarkably, the activation of Txnrd3, the reductase which expression has been considered to be limited to the genitals, was detected upon Foxn1 stimulation in keratinocytes and in the skin of Foxn1+/+ mice. We also showed that Foxn1 strongly downregulated the Ccn2 protein expression, participating in epidermal reconstruction after injury. An in vitro assay revealed that Foxn1 controls keratinocyte migration, stimulating it under normoxia and suppressing it under hypoxia. Angiogenesis assay showed that Foxn1 action reaches beyond epidermis limiting under hypoxic conditions HUVECells angiogenic properties by downregulating Vegfa expression.

Conclusion
This study showed a new mechanism in which Foxn1, along with hypoxia, participates in the

activation of antioxidant defence and controls the functional properties of keratinocytes. Future studies aiming to restore Foxn1 and its regulatory pathway in the skin of older individuals may provide new solutions for antioxidative skin protection and improvement of skin wound healing.

keywords: skin, keratinocytes, Foxn1, hypoxia, thioredoxin system

73296370389

IMAGE-BASED EVALUATION OF PASSAGE-INDUCED SENESCENCE IN HUMAN FIBROBLASTS

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[Introduction] Fibroblasts are the major cells that consist of connective tissues in animal tissues and are known as the main extracellular matrix (ECM) production cells to maintain the tissue physical properties. Although they are cells that are famous and frequently found in our body, their biological mechanisms, especially the differences from other similar fibroblastic cells are still unclear. From the tissue engineering perspective, it is an important ECM providing cells that contribute to forming solid and matured tissue with in vivo-like physical strength, therefore, for designing tough texture tissues, fibroblasts are essential cells to be included. Moreover, the function of fibroblasts is also important for understanding fibrosis formation, which is still difficult to control with conventional medical treatment. Furthermore, it is known to facilitate vascular network formations in skin-like layered tissues, therefore it is also essential for developing dermal skin models for safety test applications. However, obtaining quality-controlled fibroblasts is still difficult, because fibroblasts decrease their proliferation and factor production potencies by the continuous passages like the other normal cells, and most importantly, their quality decay timing commonly comes at sudden and is extremely difficult to predict accurately in prior. In our group, we have been reporting the effective performance of “morphology-based cell quality evaluation method (=morphometry)” as a non-invasive real-time cell quality control technique [1-3]. In this work, we investigated the morphological profiles of continuously passaged fibroblasts to define whether we can predict its quality decay only from their microscopic images.

[Method] Human normal fibroblasts (3-lots) were continuously passaged for twenty-two passages, and we collected all their time-course morphological changes in each passaged sample with the interval of 6 hours for 5 days. Moreover, we collected their total RNA’s and made RNA-seq reveal the correlation between the morphological changes and their toral expression profiles.

[Results and conclusions] From the continuously passaged fibroblast morphological information analysis, we found that their sudden decrease of proliferation potency can be predicted in prior during their passage process only by their morphological descriptors combined with AI model. Furthermore, we found that there are gene networks correlated to such morphological changes through the passage stress accumulation. Our results show a novel approach using morphological information to advance senescence research.

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keywords: Fibroblasts, passage culture, senescence

94238102088

LOSARTAN IS A POTENTIAL MODULATOR OF HYPOXIA AND ARTICULAR CHONDROCYTE HYPETROPHY

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Purpose
To investigate the effect of Losartan on articular chondrocyte hypertrophy in a monolayer culture under chemically induced hypoxia with CoCl2.

Materials & Methods
The 2nd passage of human knee chondrocytes was used to determine the cell viability in response to the treatment with Losartan or CoCl2 in the following concentrations 10-4 – 10-12 M. Chondrocytes were seeded in a 96-well plate at the density of 5×103 cells/well. Cell viability was measured after 10 days using a 0,4% Trypan blue stain. After cell viability testing to each reagent (Losartan, CoCl2), the articular chondrocytes were plated in 96-well plate at analogous density and treated every 3rd day with 10-4, 10-6, or 10-9 M concentrations of CoCl2 in the presence or absence of 10-7 M of Losartan. The control wells were treated with a cell culture medium only. Quantity of the following proteins - Collagen X (Col X) and Hypoxia-inducible factor 1 alpha (HIF-1α), were analyzed in cell lysate after 10 days using enzyme-linked immunosorbent assay (ELISA). Differences between independent variables were analyzed by Kruskal-Wallis and Mann-Whitney U-test, while Wilcoxon's signed-rank test was used for related variables. Data were considered statistically significant when $p < 0,05$.

Results
Chondrocyte viability increased in response to the treatment with CoCl2 or Losartan at lower concentrations. When cells were treated only with CoCl2, HIF-1α expression was higher compared with treatment in combination with Losartan at the same concentration of CoCl2. Col X expression was elevated when the cells were treated with CoCl2 in combination with Losartan (LC10-9 M). However, Losartan acts as a Col X inhibitor under hypoxic conditions when higher CoCl2 concentrations (LC10-4, LC10-6 M) were used.

Conclusion
Losartan reduced hypoxia in all used CoCl2 concentrations and suppressed hypertrophy of articular chondrocytes at 10-4 and 10-6 M of CoCl2 concentration.

keywords: Losartan, hypoxia, chondrocyte, hypertrophy

62825422568

STUDY OF DIFFERENT COMPONENTS FOR EFFICIENT CRYOPRESERVATION OF MESENCHYMAL STEM CELLS

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INTRODUCTION
The question of efficient preservation of cells, especially Mesenchymal stem cells (MSC) becomes more and more urgent due to the impetuous development of cell therapy in clinics. There are two main problems in the improvement of mixtures used for cells cryopreservation. First is the application of DMSO as cryoprotectant, which is known to cause different side effects, and the second is the necessity to exclude xenogeneic materials. So, the aim of this work was to minimize DMSO concentration in cryo-freeze mixture and to change fetal bovine serum (FBS) usually used in laboratories for allogeneic materials.

METHODS
MSC were isolated from human umbilical cord (UC) using explant method. After obtaining of 2nd passage, cells were frozen in different mixtures that contained from 1% to 10 % of DMSO and different additives, including trehalose, ethylene glycol and sucrose. To avoid xenogeneic material FBS was replaced with platelet lysate obtained from human plate concentrate with triple freezing. The result was evaluated by comparison of cell survival after de-freezing in one month, cell morphology and their potential for multiplication.

RESULTS
It has been shown that the best mixture for cell cryopreservation with reduced concentration of DMSO is 4% DMSO, 6% trehalose and 90%FBS. In this variant the survival rate was 94,3±0,2 compared to 95,9±0,1 in control variant with 10% DMSO. MSC morphology and growth potential were also similar. Study of the possibility to use platelet lysate instead of FBS confirmed the ability of the lysate to support cells during cryoconcervation, but this work requires more long observation as store time should be extended.

CONCLUSION
The data obtained showed that cryopreservation of MSC in the cryo-freeze mixture with a reduced concentration of DMSO provides a high survival rate of cells, does not change their biological and morphological properties. FBS in the mixture for MSC cryopreservation can be replaced by human platelet lysate.

keywords: cryopreservation,platelet lysate, cryoprotectant

20941819044

THE SPATIAL AND TEMPORAL RELATIONSHIP BETWEEN CELLULAR SENESCENCE AND THE PROCESS OF SKIN HEALING

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The term “cellular senescence” refers to an irreversible cell cycle arrest that can be caused by a variety of stressors and is accompanied by an accumulation of damage, changes in cell shape and secretory profile, and epigenetic abnormalities in primary cells. There is an accumulation of senescent cells in development, aging and tissue regeneration; all of which are conditions with increased damage and pro-growth stimuli. Importantly, while the reason for this accumulation is unclear, the senescent cells which persist in the tissue directly contribute to the pathology of age-related diseases, chronic wounds and tumor growth.

This presentation is aimed at sharing unpublished results on the kinetics of senescent cell accumulation at the sites of wounds in mice and pigs, particularly in acute mechanical wounds. BALB/c mice were wounded on the mid-back with 1 cm diameter full-thickness wounds, while minipigs were injured using a standard 6 mm biopsy punch. Wound samples were taken at various time points to characterize the kinetics of healing and senescence induction. In addition to elaborating on the temporal relationship between senescence and wound healing, we will also reveal our novel results on the spatial relationship between the site of wounding and senescence induction. Moreover, we demonstrate a clear correlation between senescence markers and features of healing such as reepithelialization and trans-differentiation of fibroblasts. Finally, we will describe the candidate proteins and pathways involved in the induction of cellular senescence at wounds.

keywords: Senescence, regeneration, aging, dermatology, cellular biology

PS27
Combined therapies for severely infected wounds accompanied with both heavy soft and hard tissue losses

83767267417

IN SILICO MODEL OF ANTIBIOTICS AND QUORUM INHIBITORS SUSTAINED RELEASE FROM THE MULTILAYER CORNEAL PATCH FOR THE MICROBIAL KERATITIS TREATMENT

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Microbial keratitis is a devastating vision-threatening ocular disease requiring immediate broad spectrum antimicrobial treatment to prevent scarring, corneal perforation, and/or endophthalmitis. In the progression stages, to reduce the burden on the immune system, keratoplasty is applied. Temporary patches usually are used until the microbial activity is over in order to preserve tissue integrity until transplantation of a complete corneal graft is possible. Antibiotic-enhanced drops are often put into the eye until the microbial activity stops. However, with the effect of tears, antibiotics are almost removed from the eye within a few minutes.
The novel strategy aims to incorporate drugs inside synthetic tectonic patches, what can enable for reducing the amount of antibiotics by suturing patches to the infected zone and releasing agents directly to the targeted cornea perforation infected area. It is also aimed to use quorum sensing (QS) inhibitors to stop the virulence of bacterial and fungal-based keratitis. Utilization of in silico methods may lead to effective designing and development of the multilayer patches with optimal parameters in terms of structure and composition.
A computational model predicting the kinetics of drugs released from particular layers of the designed corneal patch was developed. The model was based on the rabbit eyeball geometry. The model assumes the diffusional character of the drug transport, where the drug clearance is caused mainly by the aqueous humor drainage. Due to the above-mentioned fact, the vitreous body was ignored in the developed model. The aqueous humor drainage was predicted by computer fluid dynamics (CFD) modeling. The developed in silico model enables for prediction of the time-drug concentration profiles separately for ocular tissues as well as for particular layers of the corneal patch. The average drug concentration changes were assumed to result from the specific interplay between diffusive-advective drug transport and the drug release from particular layers of the designed corneal patch. The developed in silico model was validated against the experimental data on the topically administrated ciprofloxacin.

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keywords: Microbial keratitis, controlled release, corneal patch, finite element method

PS28
Emerging and future technologies for peripheral nerve regeneration

41883629928

EX VIVO EVALUATION OF NEW DECELLULARIZED PERIPHERAL NERVE-DERIVED MATRIX FOR NEURAL TISSUE ENGINEERING APPLICATIONS

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Introduction
The use of nerve autografts remains as the gold standard method for critical nerve repair. However, due to their well-known disadvantage new nerve grafts are still needed. In this sense, decellularized peripheral nerve-derived extracellular matrices (ECMs) have emerged as a promising alternative, but these processes may affect the structure, composition and biomechanical properties of the ECMs generated. In this context, the present study aimed to investigate the impact of novel chemical-enzymatic decellularization method (CE) in the histology, ECM composition and biomechanical properties of rat-derived peripheral nerves. New decellularized nerves were compared to those obtained with the classical decellularization procedure described by Sondell (SD) and native nerve (NAT).

Methods
Rat sciatic nerves of 10-mm length were decellularized by the new CE method [distilled water, 3% Triton X-100, 1% SDS, 4% SDC and enzymatic mix (RNase and DNase)] and with SD method (1,2). Afterwards, the matrices obtained were characterized by histology, electron microscopy (scanning (SEM) and transmission (TEM)) and tensile test. Native nerves (NAT) were used as control group.

Results
Conventional histology conducted with HE, showed a better preservation of the different nerve stromal layers with the use of CE method respect to SD. Moreover, none of decellularized groups presented visible nuclear remnants with 4´,6-diamidino-2-phenylindole (DAPI) staining. MCOLL staining revealed an efficient myelin removal in both groups, being these results confirmed by SEM and TEM analyses. Furthermore, electron microscopy confirmed a complete decellularization process with both protocols but collagen organization was better preserved in CE as compared to SD. Finally, the tensile test showed no significant differences ($p>0.05$) in the

strain at fracture and Young´s modulus between the matrices obtained by the new CE method and SD technique, being both closely comparable to NAT control group.

Discussion & conclusion
This ex vivo study demonstrated that the new CE method is a promising alternative to efficiently remove the cellular content from peripheral nerves. When compared with SD classical procedure, the new method showed better histological and ultrastructural features with a better preservation of the collagen network. From the biomechanical point of view, this study confirmed that this new method did not considerably affect the overall biomechanical properties of the matrices generated. Finally, further in vivo studies are needed to elucidate the regenerative potential of these new biomedical products.

Fundings
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keywords: Decellularized peripheral nerve, Tissue engineering, Extracellular matrix, natural-derived hydrogel

94238126677

IN VIVO HISTOLOGICAL AND HISTOMORPHOMETRICAL EVALUATION OF A NOVEL DECELLULARIZED PERIPHERAL NERVE ALLOGRAFT IN RATS

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Introduction: As a reliable alternative to autografts, decellularized peripheral nerve allografts (DPNAs) maintain the complex natural structure of native nerves minimizing the immune response. However, there is currently a lack of decellularization methods for peripheral nerve that remove cells minimizing any adverse effect in extracellular matrix (ECM) that overcoming the autograft results. The aim of this study was to evaluate the in vivo regeneration potential of a novel chemical-enzymatic decellularization method (CE) for peripheral nerves in comparison with the decellularized classical control group Sondell (SD) and the autograft technique (AUTO). Methods: Sciatic nerves from Wistar rats were decellularized by the new CE method [distilled water, 3% Triton X-100, 1% SDS, 4% SDC and enzymatic mix (RNase and DNase)] and with SD method (1). Afterwards, they were used to repair a 10-mm sciatic nerve gap and compared to autograft technique (1) and native nerves as control group (CTR). After 15-weeks the nerve tissue regeneration was evaluated by using histological (HE, MCOLL and immunohistochemistry) and histomorphometrical methods.

Results: Histologically, hematoxylin-eosin (HE) showed an active regeneration process in all operated animals, being more dispersed in decellularized groups in comparison with AUTO group. MCOLL histochemical method showed a higher degree of myelinization in AUTO and CE groups as compared to SD group. In addition, MCOLL revealed signs of fibrotic response around the regenerated tissue in SD group. Nerve regeneration was confirmed by the positive immunoreaction for Schwann cells (S-100) and regenerated axons (neurofilament (NFL)) in all groups. However, overall regeneration was more organized in AUTO group followed by CE groups and finally SD. Histomorphometrical analysis showed that the new decellularized nerves obtained with CE protocol presented significant superior density and total number fiber results ($p< 0.05$) than the classic SD group. Moreover, CE group showed comparable histomorphometrical results than the AUTO group ($p>0.05$). As expected histomorphometrical values were not comparable to those observed in healthy nerves.

Discussion & conclusion: This in vivo histological study revealed promising results with the use of the novel decellularized allografts obtained with CE method. The CE method resulted in a superior regeneration histological profile than the classical method of SD. These results were comparable, but not superior, to the gold standard AUTO group. Finally, more studies are still needed to determine the efficacy of these new products in the treatment of long nerve gaps at the histological, molecular and functional levels.

Fundings: Supported by the Spanish “Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, Ministerio de Economía y Competitividad (Instituto de Salud Carlos III)”, Grant No FIS PI20-0318 co-financed by the “Fondo Europeo de Desarrollo Regional ERDF-FEDER European Union”; Grant No P18-RT-5059 by “Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, España”; and Grant A-CTS-498-UGR18 by “Programa Operativo FEDER Andalucía 2014–2020, Universidad de Granada, Junta de Andalucía, España”, co-financed by the ERDF-FEDER, the European Union.

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keywords: Decellularized peripheral nerve, Tissue engineering, Nerve repair, Histology and histomorphometry, In vivo nerve regeneration

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SILK-SILK CONDUITS FILLED WITH NATIVE SPIDER SILK FIBERS SUCCESSFULLY PROMOTED NERVE REGENERATION IN A 10 MM SCIATIC NERVE DEFECT IN RATS

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Introduction:
The surgical repair of nerve transection injuries remains a challenging task and often results in unsatisfactory functional recovery. If a direct coaptation is not possible, the current gold-standard is the use of an autograft. However, the availability of autologous nerve tissue is limited and the harvest of a donor nerve entails functional loss and possible donor site morbidity. In the search for alternatives, different synthetic and biological materials are currently tested to bridge nerve gaps. Recent studies supported silk as promising material for tissue engineering and the development of artificial nerve conduits. In addition, nerve conduits that contain an internal framework as guiding structures could enhance a directed axonal re-growth. Spider silk possess excellent mechanical properties such as an adequate tensile strength, long-term degradability and a non-immunogenic nature, which support their use as promising conduit filling material. In this study, we investigated the performance of a silk fibroin-based conduit filled with spider silk fibers to bridge a 10 mm sciatic nerve defect in rats.

Methods:
In 18 male Sprague-Dawley rats, a 10 mm piece of the sciatic nerve was resected and immediately bridged with 1) autografts (control group, n=6), 2) empty silk conduits (experimental group one, n=6), and 3) silk conduits filled with spider silk fibers (experimental group two, n=6). Walking track analysis was performed for each animal prior to surgical intervention and every 14 days over a course of 14 weeks. Functional recovery was evaluated by calculating the sciatic functional index (SFI) according Bain et al. At the endpoint, animals were sacrificed and the nerves were harvested to assess axon re-growth and myelination by histomorphometric as well as immunofluorescence analyses on paraffin sections.

Results:
The walking track results showed that there was no statistical difference in the mean SFI of animals treated with the autograft or the silk fiber containing silk conduits. Moreover, the immunofluoresence stainings of nerve sections illustrated a similar pattern of regenerated nerve tissue in sections of autografts and filled silk conduits, while a less advanced nerve regrowth was seen in the samples containing empty silk conduits. The histomorphometric parameters displayed a similar number of myelinated axons in the autografts and filled silk conduits. Additionally, the mean axon area was comparable between the autograft and the silk conduit filled with spider silk. However, the mean myelin area was the largest in the autograft group.

Conclusion:
Taken together, our study demonstrated that the functional recovery of a 10 mm sciatic nerve defect bridged with silk conduits containing spider silk fibers as internal guiding structure was comparable to and autologous nerve grafts.

keywords: peripheral nerve regeneration, spider silk

PS29
Engineered viscoelasticity in cell and tissue engineering

83767284609

ENGINEERING AND DESIGN OF BIOMIMETIC VISCOELASTIC HYDROGELS

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Introduction
Studies investigating cell response to substrate viscoelasticity are usually based on hydrogels where both viscous and elastic properties are altered at the same time [1-2], so the two effects cannot be decoupled. We engineered agarose-based hydrogels with a constant equilibrium elastic modulus and different characteristic relaxation times that can be modulated varying the liquid phase viscosity without modifying the crosslinking of the solid network [2]. The agarose gel properties were optimised for the culture of adipose-derived mesenchymal stem cells (ADSCs) to understand if they are able to sense and respond to viscoelastic substrates with different viscous properties.

Methods
0.5% w/v agarose gel were fabricated using aqueous solutions with increasing dextran concentrations (0, 3, 4 % w/v), and hence viscosities. Mechanical properties were investigated using the epsilon-dot method [3]. ADSC (50.000 cells/cm2) were cultured for 7 days on the gels with 2 (high τ) and 4% (low τ) w/v dextran coated with 5% w/v gelatin and in 96-well multiwell plates ($\tau \rightarrow \infty$) as controls. Bright field images were acquired at day 3 and 7 (Olympus). Then, cells were fixed and stained with DAPI and rhodamine-conjugated phalloidin. Immunostaining was also performed to assess the presence of CD45 (negative marker for cell stemness). Images were acquired with a confocal microscope (Nikon A1, Japan).

Results
The gel instantaneous elastic moduli (Einst) and relaxation time (τ) decreased significantly with increasing dextran concentration, while the equilibrium elastic modulus (Eeq) did not vary significantly. Moreover, Eeq was in the optimal range to mimic the mechanical properties of the stem cell niche ($\cong$ 3 kPa [2]). Bright field imaging at day 3 and 7 allowed us to investigate morphological differences between cells on the two substrates. In the controls, we observed high cell spreading; the formation of round shaped cell clusters and only few elongated cells were noted in the 2% gels. Finally in the 4% gels, we observed higher cell spreading with respect to the 2% gels. The cell area was significantly lower in the gels 2% gels with respect to both the 4% gels and the controls. This suggest that cells are able to sense differences in the substrate viscous behaviour, and that, in the gels with the same elastic behaviour, cell spreading increase with decreasing relaxation time. Moreover, a significantly lower CD45 expression in the gels with respect to the control indicate cell tendence to maintain stemness.

Conclusions
Hydrogel viscoelasticity was engineered allowing the study of cell viscoelastic mechanotransduction as a function of gel viscous behaviour. The design of materials with well-defined relaxation behaviour, able to consider the different time scales involved (cell sensing time, substrate relaxation time and observation time), will be essential for generating biomimetic viscoelastic materials for regenerative medicine applications [3].

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keywords: viscoelasticity, hydrogels, mechanotransduction, mesenchymal stem cells

PS31
Extracellular vesicles – next generation tool for diagnostics and regenerative medicine

73296306444

CHARACTERIZATION OF EXTRACELLULAR VESICLES FROM PORCINE, CANINE, AND HUMAN NOTOCHORDAL CELL-CONDITIONED MEDIUM

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Introduction
Low back pain (LBP) episodes are common and affect everyday life. A major cause of chronic LBP is intervertebral disc degeneration. Notochordal cells (NCs), the juvenile disc cells only present in young individuals and not in degenerated IVDs, possess regenerative potential that could be exploited for therapeutic approaches. Previous work indicated that porcine NCs secrete extracellular vesicles (EVs) that may mediate this effect (1). To ensure that the observed effects in biological studies are associated with EVs and not associated with soluble bioactive molecules, characterization of the EV preparation is essential (2). This study aims to perform EV characterization of multiple species using a bead-based western technology to identify NC-derived EV-associated protein markers.

Methodology
NC-conditioned medium (NCCM) was generated by culturing NC-rich tissue of porcine, canine, and human origin. EVs were isolated through differential centrifugation followed by size exclusion chromatography (SEC). EV containing fractions were identified based on protein content and pooled for analysis. The SEC fractions from porcine NCCM were pooled in sets of three and subjected to analysis. NC-derived EVs were characterized using DigiWest technology, a high-throughput bead-based multiplex platform (3).

Results
Using the DigiWest technology, a panel of 33 proteins was determined in NCCM-derived EVs. The analysis of porcine and canine NCCM-derived EVs revealed the presence of 12 EV-associated protein markers in common. In human NCCM-derived EVs, only two proteins (MFGE8 and fibronectin) were identified, most probably due to technical limitations related to low starting protein quantities. These two proteins were also present in porcine/canine NCCM-derived EVs. Possible co-isolated proteins that were detectable in control tissue samples were not detected in the EV-fractions of these tissues.
In addition, the DigiWest platform was used to detect EV-associated proteins in SEC fractions containing relatively small protein quantities (0.9-9 µg input). An enrichment in EV markers (CD9, TSG101, flotillin 1, and HSPA8) was seen in fractions 7-12 compared to later fractions, as expected based on the reported EV-marker profiles.

Conclusion
Altogether, for porcine and canine NCCM-derived EVs, several transmembrane, GPI-anchored, and cytosolic proteins were identified, which is recommended for EV characterization according to the standards of the International Society for Extracellular Vesicles (2). Additionally, the EV samples were devoid of some non-EV-associated tissue proteins that could be co-isolated. Based on these results, a panel of 19 proteins was composed to characterize NC-derived EVs from different species.

The identification of EVs in separate SEC fractions has major challenges with, amongst others, low protein quantities in the fractions and the need for parallel identification of multiple protein markers for proper EV characterization (2). However, the DigiWest technology has been shown to successfully identify multiple EV-associated proteins in a sample containing small protein quantities. It may therefore be used to identify and characterize EVs in SEC fractions.

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keywords: Intervertebral disc degeneration, DigiWest technology, Extracellular vesicles, Protein markers

73296321555

COMPARISON OF EXTRACELLULAR VESICLES PRESENT IN BONE, BLOOD, AND EXTRACELLULAR MATRIX

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Introduction: The term "extracellular vesicle" (EV) is broadly used to describe naturally released cellular vesicles with a lipid bilayer and without a nucleus. EV represent a heterogeneous population that is generally categorized into subpopulations based on characteristics such as size, biogenesis, function, and composition. The field of extracellular vesicle (EV) research is growing rapidly, but current metrics to delineate differences between vesicle subpopulations are limited. Recently, a type of EV embedded within the extracellular matrix of soft tissues termed matrix-bound nanovesicles (MBV) has been described but defining characteristics of MBV relative to other types of EV have not been established. The present study compared three distinct subpopulations of EV, namely mineralization-competent matrix vesicles (cMV), exosomes (Exo), and MBV.

Methods: MBV were isolated from mouse skeletal muscle that was decellularized, minced, and digested enzymatically to release the MBV from the extracellular matrix. cMV were isolated from the matrix of mineralizing mouse 17IIA11 pre-odontoblast cells. Exo were isolated from mouse plasma. The three types of vesicles were quantified using NanoSight Nanoparticle Tracking Analysis. The vesicle size, physical characteristics, protein cargo, miRNA cargo, and lipid membrane composition were compared. The immunomodulatory activity on naïve and pro-inflammatory bone-marrow derived macrophages was also evaluated.

Results: NanoSight and transmission electron microscopy (TEM) showed similar size and morphology of each vesicle type. Characterization of protein cargo showed that MBV contain low levels of proteins commonly associated with both Exo and cMV, including CD63, CD81, Annexin V, and alkaline phosphatase. Each vesicle type was associated with a unique profile of cytokine cargo. Lipidomic analysis showed clear differences in membrane composition of MBV relative to both Exo and cMV. The miRNA cargo of MBV and cMV were more similar while Exo-associated miRNA were highly distinct. Characterization of the immunomodulatory activity of each vesicle type showed that cMV induce pro-inflammatory activation of macrophages, while MBV induced a more anti-inflammatory phenotype.

Conclusion: The present study shows that despite similar physical characteristics, these three types represent distinctly unique subpopulations of EV. These results inform metrics for proper categorization of EV and guides choice of EV based on desired functional parameters.

keywords: extracellular vesicle, matrix bound nanovesicle, exosome, calcifying matrix vesicle, immunomodulation

41883605644

DEVELOPING OF HUMAN IPS-DERIVED CARDIAC CELL LINE IN VITRO MODELS FOR STUDYING AN IMPACT OF EXTRACELLULAR VESICLES IN HEART REPAIR - PRELIMINARY REPORT

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Introduction
Since the cardiac tissue possesses limited regenerative capacity, cardiovascular diseases still remain one of the most common causes of death worldwide and their currently existing treatment approaches, including cell-based therapies, are still unsatisfactory. Thus, new therapeutic strategies need to be developed, which is a one of the challenges of modern biomedical sciences. Recently, a promising approach utilizing extracellular vesicles (EVs) has been proposed.
EVs as lipid bilayer-coated particles carrying bioactive cargo, are released from different types of cells, including stem cells and were shown to play an important role in cell-to-cell communication. Thus, growing data demonstrates that EVs may serve as potential new-generation cell-free therapeutic agents in the field of tissue regeneration. Moreover, it has been shown that human induced pluripotent stem cell (hiPSC)-derived EVs (hiPSC-EVs) may contain and transfer miRNA molecules that promote regeneration of cardiac tissue after the injury. However, since a detailed mechanism of this phenomenon and the exact role of miRNA remain unknown, in the current study we aimed at developing an in vitro models to investigate the role of selected miRNA clusters in pro-regenerative activity of hiPSC-EVs.

Methodology
Based on pluripotent capacity of hiPSCs, we differentiated those cells into three cardiac cell lines: cardiomyocytes (CM), cardiac fibroblasts (CF) and cardiac endothelium (CE), that would serve as target cells for hiPSC-EVs in further functional in vitro assays. Due to the lack of well-established and universal differentiation protocols, we compared several approaches and experimental conditions on two different hiPSC lines. Next, the differentiation efficacy and stability of cell phenotype over further passages were assessed by microscopic observations, as well as flow cytometry and gene expression analyses (RT-qPCR).

Results
Our preliminary results demonstrated the successful differentiation of both tested hiPSCs lines into three desired cardiac cell lines, as indicated by changes in the morphology, followed by expression of specific lineage markers. However, we observed different efficacy between tested hiPSC lines and experimental conditions, which confirms the complexity of the differentiation process and the need for further tailoring of tested protocols.

Conclusions
In conclusion, we successfully established target cardiac cell lines as an in vitro models, which may be suitable for the investigation of miRNA role in heart regeneration mediated by hiPSC-EVs. However, due to the observed variations between selected experimental conditions,

further studies are required to select the preferable hiPSC line as well as the most optimal differentiation approaches.

Acknowledgements
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keywords: extracellular vesicles (EVs). human induced pluripotent stem cells (hiPSC). miRNA. cardiac regeneration.

52354583169

IMPACT OF MESENCHYMAL STEM CELLS DERIVED LAMININ-BINDING EXTRACELLULAR VESICLES ON SCHWANN CELLS AND IN PERIPHERAL NERVE REGENERATION PROCESSES

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Injuries to peripheral nerves is a common health problem, often resulting in sensory and motor dysfunction in respective parts of the body. One of the most important facilitators of the regeneration process are Schwann cells (SCs), which basement membrane is chiefly comprised of laminin. Extracellular vesicles (EVs) are considered playing an important role in the intercellular communication and transfer of biological information. Especially, Mesenchymal stem cell-derived EVs (MSC-EVs) have been identified as a promising novel therapeutic option due to their potential function as target-directed drug delivery vehicles. However, the precise delivery of EVs upon administration is still a huge challenge due to the accumulation of EVs at off-target sites. To remedy this problem, this study focuses on the production of laminin-binding EVs derived from MSCs by modification of the large extracellular loop (LEL) of the overexpressed EV surface marker protein CD81, from the tetraspanin protein family, to increase their binding affinity to laminin.
Specific CD81-LEL sequences are cloned into lentiviral vectors encoding the expression cassette for full-length CD81 proteins fused with eGFP or Akaluciferase under the control of human cytomegalovirus promoter. Stable cell lines are obtained upon transformation of Wharton's Jelly MSCs (WJ-MSCs). EVs derived by WJ-MSCs are further isolated and characterized by NTA. Their uptake by SCs is evaluated by detecting the emitted fluorescence with FACS.
Our results obtained from NTA demonstrate the successful cloning and production of laminin-binding EVs. The positive tracking of fluorescence with FACS verifies their binding affinity on rodent laminin and their increased uptake and pro-regenerative effect on SCs.
This study represents a substantial advance for further investigation on EVs regarding their influence on peripheral nerve regeneration processes.

keywords: Extracellular Vesicles, Peripheral nerve regeneration, Schwann cells

31412726466

IMPROVEMENT OF HUMAN KERATINOCYTE CELL CULTURE PROTOCOLS FOR USE IN ORAL MUCOSA TISSUE ENGINEERING

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Introduction: Development of bioartificial substitutes of the human oral mucosa and palate is strictly dependent on the availability of human keratinocyte cells cultured from small tissue biopsies1. Although cell culture methods have been improved over the last years, epithelial cells typically show low proliferation rates and cell culture protocols should be improved for an efficient use in Tissue Engineering. A promising strategy is the use of mesenchymal stem cell (MSC)-derived secretome to increase cell culture. Secretomes contain high amounts of growth factors, cytokines, proteases, transcription factors and other molecules involved in cell adhesion, migration, proliferation, and differentiation that may improve epithelial cell culture2. In this work, we evaluated several types of secretomes obtained from different MSC sources to evaluate their effect on human keratinocyte cell proliferation.

Methodology: To generate primary cell cultures of human adipose tissue, dental pulp, and human umbilical cord, biopsies were obtained and enzymatically digested. Isolated cells were collected by centrifugation and cultured under standard cell culture conditions. Afterwards, the medium containing the products released by each type of MSC was harvested and centrifuged for 10 minutes to eliminate all cellular debris and apoptotic bodies, followed by filtering. Subsequently, human keratinocytes were cultured in the presence of each type of secretome at the concentration of 0%, 25%, 50%, 75% and 100% for 24, 48, 72 and 120 hours. Cell viability and cell proliferation were evaluated in each experimental condition.

Results: Our results revealed that more than 80% cell viability in all study groups, with higher survival at the longest exposure times. Regarding proliferation, results were comparable to controls at the shortest follow-up times, but a significant increase was found at 72 and 120h of incubation in the presence of the different secretomes, with higher results in the case of secretomes obtained from dental pulp and human umbilical cord MSC.

Conclusion: Our data support the idea that MSC-derived secretome is not toxic for epithelial cell culture, and cells cultured with this product were highly viable. In addition, its use seems to increase cell proliferation, especially after long exposition times. These results open de door to the future use of these types of secretomes to obtain efficient cell cultures of human keratinocytes for use in oral mucosa and palate tissue engineering.

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keywords: Human Mesenchymal Stem Cells, Secretome, Oral Mucosa, Palate

20941830248

LIPOSOMAL VITAMIN C AS AN ATRACTIVE ALTERNATIVE FOR ASCORBIC ACID SUPPLEMENTATION IN CELL CULTURE

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Introduction: Vitamin C (ascorbic acid) is a potent antioxidant and a crucial co-factor of many enzymes, including Fe2+ and alpha-ketoglutarate dependent dioxygenases such as TET (ten-eleven-translocation) proteins involved in active DNA demethylation. TET enzymes (TET1, TET2 and TET3) are able to oxidize 5-methylcytosine to 5-hydroxymethylcytosine and subsequently to 5-formylcytosine and 5-carboxycytosine - modifications involved in epigenetic regulation of gene expression. However, in order to maintain enzymatic function of those enzymes, after each step of oxidation, catalytic Fe ion in their active center must be restored to 2+ state by vitamin C. Therefore, in many studies involving epigenetic make-up, gene expression, cell maturation and differentiation supplementation of the vitamin C seems to be indispensable for obtaining most accurate and reliable data. However, vitamin C in aqueous solutions is unstable, quickly degrades and may change pH of culture medium. Moreover, in many cells the effect of vitamin C supplementation may be affected by abnormalities in the functioning of vitamin C transporters. Therefore, we have investigated effects of different forms of vitamin C on enzymatic activity of TET enzymes, in order to determine which of them can be an alternative to supplementation with crystalic ascorbic acid.

Methodology: HAP1 and MDA-MB-231 cells were cultured in accordance to manufacturer protocols and exposed for 24 hours to 100 μM of: ascorbic acid, sodium ascorbate, 6-O-palmitoyl-L-ascorbic acid, liposomal sodium ascorbate and “empty” liposomes (last two provided by Lipid Systems). Control cells were treated with volumetric equivalent of saline. DNA from harvested cells was isolated by phenolic extraction and subsequently enzymatically hydrolyzed to obtain nucleosides. Enzymatic activity of TET proteins characterized as the levels of their products in cellular DNA - was measured by two dimensional ultraperformance liquid chromatography with tandem mass spectrometry (2D-UPLC MS/MS).

Results: In both cell lines we have observed significant increase in the levels of 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine after incubation with each form of vitamin C when compared with untreated cells and cells exposed to “empty” liposomes. There were no significant differences between “empty” liposomes and control cells. Moreover, there were no significant differences in the levels of TET products between each form of vitamin C.

Conclusions: Liposomal form of vitamin C and 6-O-palmitoyl-L-ascorbic acid stimulate activity of TET proteins in the same manner as a free ascorbic acid, but are more stable in cell

culture conditions. However, 6-O-palmitoyl-L-ascorbic acid is highly hydrophobic and must be dissolved in organic solvents that may potentially affect cell viability or their phenotype. Taking this into consideration, liposomal vitamin C seems to be a better alternative for vitamin C supplementation, as it is as efficient as ascorbic acid in stimulating enzymatic activity of TET proteins (and potentially other dioxygenases), is well soluble in aqueous solutions and does not change the pH of the culture medium. Moreover, due to passive transport of liposomes to the cells, effect of liposomal vitamin C supplementation is potentially unaffected by the expression patterns or mutational status of vitamin C transporters.

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keywords: vitamin C, epigenetics, TET proteins, supplementation, cell culture,

PS32
Extracellular vesicles for soft tissue repair

20941824705

ADIPOSE STEM CELL SECRETOME AS A POTENTIAL TREATMENT FOR URETHRAL FIBROSIS

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Urethral fibrosis affects 1 out of 1000 individuals within their lifetime, most of those affected being male. It shares the hallmarks of other types of fibrosis (e.g., liver or heart), namely, the activation of myofibroblasts, overabundance of collagen and the spread of scarring from the initial focus to the surrounding tissue, leading to organ failure. It can be caused by trauma, infection and chronic inflammation; in the case of urethral fibrosis, the cause is occasionally iatrogenic. Currently, the most effective treatment of fibrosis is organ or tissue transplantation (if possible); there are several pharmaceuticals used as well, however, the options are limited and new ways of treating fibrosis need to be investigated. The secretome of stem cells has been explored for treating various diseases; for example, it has shown promise in the case of treating idiopathic pulmonary fibrosis. Nevertheless, both the causes of fibrosis and the molecular mechanisms that could be affected to prevent or treat this illness have not yet been elucidated. Thus we decided to investigate the effect the stem cell secretome has on myofibroblasts, and whether this could reveal new ways of fibrosis treatment.

Primary human myofibroblasts (HMF) were isolated from scarred urethral tissue and confirmed to present the α-smooth muscle actin (αSMA) myofibroblast marker. Human adipose stem cells (HASC) were isolated from adipose tissue and shown to be CD44 and CD90 positive. Their differentiation multipotential was also confirmed. WPMY-1 prostate cancer stromal myofibroblasts (ATCC) was chosen as a control cell line. To better recapture the fibrotic environment found in the affected tissues, the myofibroblast cells were cultivated on PDMS coated with fibronectin. The effect of the HASC conditioned medium (HASC-CM) was compared to the effect of treatment using a common antifibrotic drug at a therapeutic concentration. Treatment effects were analyzed by performing Western blot for αSMA and latent TGFβ, another player in fibrosis development. Moreover, the gene expression levels of fibrosis-related and TGFβ signalling pathway genes were quantified.

The myofibroblasts cultivated on fibronectin-coated PDMS exhibited significant differences in fibrosis-related gene expression levels compared to cells cultivated on tissue culture plate surface, confirming the importance of tuning the cultivation surface to the natural cell environment. Both the HMF and WPMY cells showed clear differences after treatment with

either HASC-CM or antifibrotic drug, with the increase of latent TGFβ (both treatments) and changes in αSMA amount (decreased in the drug group, increased in CM group). The gene expression changes after treatment were also evident: cells expressed more Mmp1 (collagenase I, an anti-fibrotic marker) and less Ctgf (pro-fibrotic marker) after treatment with CM. Lastly, as treatment with CM did not reduce Tgfb expression, our findings point the anti-fibrotic effect may be elicited through reduction of Rock1 and Rock2 expression.

We summarize that the adipose stem cell secretome reduces the amount of fibrotic markers in myofibroblast culture, although through different mechanisms than conventional anti-fibrotic drugs.

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keywords: Myofibroblasts, secretome, human adipose stem cells

83767208604

EXTRACELLULAR VESICLES DERIVED FROM AMNIOTIC FLUID MESENCHYMAL STEM CELLS SELECTED BY SKIN TISSUE TYPE MARKERS REDUCE INFLAMMATION

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Introduction
One of the main stages during wound healing is inflammation. There are indications that extracellular vesicles (EV) from mesenchymal stem cells (MSC) can ameliorate inflammation during this process. MSC have immunomodulatory properties and can induce regeneration. MSC from various sources have been used in wound healing studies. Moreover, several studies have demonstrated that conditioned media from MSC cultures have similar, or even higher, regenerative capacity than the MSC themselves when applied to wounds. Thus, the regenerative capacity of MSC could be due to their paracrine activity, including EV. EV contain molecules including nucleic acids and proteins and the composition depends on the cell type and its physiological conditions. MSC derived from term Amniotic Fluid (TAF) are neonatal and can be propagated to extremely high amounts for use in cell therapy. Furthermore, sorting strategies using tissue-specific markers can prepare a sorted population of cells that can be used for each tissue-type. EV derived from skin-specific TAF-MSC are highly interesting for wound healing applications.

Methodology
RNASeq data from TAF-MSC clones was used to identify skin-specific markers present on these MSC. Several prospective skin markers were tested by flow cytometry on cultured TAF-MSC. One of these markers was used for cell-sorting using Tyto MACSQuant cell sorter and the positive cell population was expanded to final product stage (CutiStem). The secretome of CutiStem cells was collected after starvation. Extracellular vesicles were purified by ultra-centrifugation. Nanoparticle tracking (NTA) measurements were used to assess size and concentration by Zetaview. MACSPlex was used for multiplexed flow cytometry marker analysis. An immunomodulation assay was used to test action on the NFkB pathway. THP-1 dual cell monocytic cell line was constructed by stable integration of two inducible reporter constructs. This THP-1 cell assay was assessed by monitoring the activity of SEAP, the reporter protein. Samples were treated with LPS (positive control), dexamethasone (negative control), or a combination thereof.

Results
Several prospective markers were tested of which one was used for skin-MSC sorting. Sorting at passage 2 increased positivity of this marker from 17% to 88%. The positive fraction was further propagated until passage 7 (CutiStem). CutiStem cells were cultured to 70% confluence whereafter growth medium was exchanged to starvation medium. Secretome was collected after 72 h of starvation. EV were purified by ultracentrifugation and analyzed. NTA revealed high yield of pure EV with mean particle size of 210 ± 5 nm. MACSPlex and flow cytometry showed high presence of exosome markers CD9, CD24, CD29, CD63 and CD81 and MSC markers CD105 and CD146. NFkB pathway activity was assessed by THP-1 assay, indicating that EV derived from CutiStem reduce inflammation. The unpurified secretome did not reduce inflammation, indicating that the EV fraction is responsible for the immunomodulatory activities and not the free proteins of the secretome.

Conclusion
One marker was found to be a good skin-specific marker for sorting of TAF-MSC using the Tyto MACSQuant cell sorter instrument. Sorted cells were further propagated and the secretome was collected for further analysis. EV derived from skin-sorted TAF-MSC (CutiStem) reduce inflammation.

keywords: amniotic fluid, tissue specific, EV

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INFLUENCE OF INTERLEUKIN -10 ON EFFECTS ELICITED BY HUMAN MSC TRANSPLANTATION IN MICE WITH EXPERIMENTAL INFLUENZA VIRUS - INDUCED PNEUMONIA

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Introduction. Interleukin 10 (IL-10) is anti-inflammatory cytokine that plays central regulatory role in immune-mediated inflammation caused by viral infection. The purpose of this work was to study the action of IL10 on the efficiency of cell therapy in a mouse model of influenza pneumonia.

Methodology. We used an experimental model of influenza pneumonia obtained by intranasal infection of white non-inbred mice weighing 14-18 grams with influenza virus A / FM / 1/47 / H1N1. The virus was adapted to the lung tissue of mice, infectious titer - 4.0 lg ID50, hemagglutinin titer 1:256 GAO/0.2 ml. The recombinant human interleukin-10 expressed in E.coli was used in the study. For cell transplantation human umbilical cord MSCs were used. Transplantation have been performed with native MSCs, MSCs pretreated with human interleukin 10, and MSCs transfected with a plasmid containing the IL10 gene. The study used several groups of animals that were injected with different doses of IL-10 and 200,000 MSCs. Mouse lung morphometry and histology have been studied.

Results. It was shown that the efficiency index of intravenous and intraperitoneal administration of IL-10 (concentration 1mkg/100 mkl) was 40-60%. Survival rate of infected animals after introduction of IL-10 followed by transplantation of MSCs native, transfected and primed with IL-10 have been increased to 80%. The infectious titer of the virus in the lung tissue decreased by 2-4 lg ID50 compared to control animals. There were no signs of exudative serous-hemorrhagic inflammation in the surviving experimental animals in the immediate and long-term periods (8-22 days).

Conclusion. As a result of the studies, it was shown that intravenous administration of IL-10 at a concentration of 1mkg/100 mkl and transplantation of primed and transfected MSCs exhibited a substantial therapeutic effect, increased the survival rate of mice up to 60-80%, and inhibited the reproduction of influenza virus in the lung tissue by 2-4 lg ID50.

keywords: Interleukin 10, human MSCs, influenza virus- induced pneumonia

62825445549

MESENCHYMAL STROMAL CELLS-DERIVED EXTRACELLULAR VESICLES: FROM 2D TO 3D

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Introduction
The secretion of therapeutic factors and extracellular vesicles (EVs) by mesenchymal stromal cells (MSCs) gained much interest for their benefits in the regenerative medicine field. Indeed, the in vitro multipotency of these cells and the secretion of both angiogenic and immunomodulatory factors suggest a role in tissue repair and regeneration. However, during culture in monolayer, MSCs rapidly lose the expression of key transcription factors associated with multipotency and self-renewal, as well as the ability to produce functional paracrine factors. In this scenario, it is essential to find alternative culture methods, which more faithfully simulate the physiological characteristics of these cells. It has been reported that 3D cultures allow cells to be cultured for a longer period, without losing their stemness, and mimicking the situation that occurs in vivo. The aim of this work was to evaluate if 3D culture can determine also an effect on EVs secretion.

Methodology
MSCs were obtained from bone marrow aspirates. Cells were cultured until passage 2, then spheroids were created using the 3D Petri Dish® system. It consists of a tiny 96-well plate of 2% agar, which does not allow the cells to adhere, stimulating their aggregation. Cell were also cultured in monolayer as control. Cell in both conditions were analysed by real-time PCR and flow cytometry. EVs were also isolated from conditioned media. They were characterized through different methods including nanoparticle tracking analysis, western blot, non-conventional flow cytometry, according to MISEV 2018.

Results
Spheroid cultures from MSCs showed a drastic decrease in dimension after first days in culture, due to a cytoskeleton reorganization which led to a morphology change. Cells in 3D lose the classical fibroblastic spindle shape morphology, becoming polygonal. Interestingly, despite maintaining the stem characteristics, MSCs grown in 3D partially modify their surface phenotype, expressing CD31 and CD34. These are surface markers normally negative in mesenchymal lineage and positive in hematopoietic cells. Analysing the EVs released by MSCs cultured in 2D and 3D, we observed that spheroid cultures exhibit a higher release of EVs, however maintaining similar characteristics to the EVs derived from MSCs in 2D.

Conclusions
The use of this culture strategy could be a valid alternative to traditional culture methods for EVs production by MSCs. As future perspective, a dynamic system could be used in order to better mimic the in vivo environment.

keywords: extracellular vesicles, spheroids, 3D, MSCs

73296374169

THE BIOACTIVITY OF PLATELET-RICH FIBRIN CONDITIONED MEDIUM ON ZOLEDRONATE-INDUCED ORAL KERATINOCYTE TOXICITY IN VITRO

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Introduction:
Medication-related osteonecrosis of the jaw (MRONJ) is an adverse effect of bisphosphonate therapy which is characterised by the loss of overlying soft tissue and exposure of necrotic bone in the oral cavity1, affecting the quality of life of patients2. The lack of effective treatment approaches3 leads to the development of alternative therapies including platelet-rich fibrin (PRF) applications. Clinical studies have shown that PRF can improve the healing of MRONJ wounds, but the evidence is limited and the mechanisms of PRF on the healing process have yet to be explained. Thus, the aim of this in vitro study was to investigate the impact of PRF in the form of conditioned medium on oral keratinocyte activities in the presence of zoledronate (ZA), the most potent nitrogen-containing bisphosphonate.

Methodology:
Blood samples from healthy volunteers were taken and processed to obtain a liquid formulation of PRF (Liquid-PRF). Collected PRF was incubated with cell culture medium for 3 days at 37oC to prepare the conditioned medium (100%). Oral keratinocytes were treated with different conditioned medium derived from liquid-PRF or in combination with 10 μM ZA. To determine the role of PRF on the wound healing process, metabolic activity, proliferation and migration of keratinocytes were investigated.

Results:
We found that ZA decreased the metabolic activity and proliferation of oral keratinocytes after 72 hours. The addition of PRF-conditioned medium produced a slight increase in cell metabolism. Interestingly, treatment with the highest concentration (50%) of PRF-conditioned medium significantly increased the proliferation index of ZA-treated keratinocytes. At 24 hour, ZA inhibited keratinocyte migration across the gap, representing a clinical wound. All concentrations of PRF-conditioned medium produced a complete closure of the cell-free gap when compared to ZA-treatment.

Conclusion(s):
These findings suggest that PRF could play a part in the healing process of ZA-induced toxicity by promoting cell migration and proliferation. Future work on the effects of PRF using 3D tissue models are required to confirm the potential bioactivity of PRF on the healing of soft tissue wounds in MRONJ patients.

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keywords: MRONJ, Keratinocytes, Zoledronate, Proliferation, Migration

PS33
From Bench-to-Bedside: Translating 3D Printing Applications in Tissue Engineering and Regenerative Medicine

31412712467

3D-PRINTED PCL-BASED GYNAECOLOGICAL MESHES: A NEW STRATEGY TO ENHANCE TISSUE-MESH INTEGRATION

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INTRODUCTION
Pelvic floor dysfunctions (PFDs) are a group of disorders affecting one-third of women worldwide and 50% of women above 55, with pelvic organ prolapse (POP) being the most common. Nowadays, different approaches are used to treat POP, including a surgical approach that consists in the placement of a polymeric mesh. Unfortunately, the employment of meshes has been often associated with the development of significant side effects (e.g. fibrosis, infections, stress shielding). Moreover, in accordance to the United States Food and Drug Administration (FDA) updated guidelines, current marketed products lack of customisability and are unable to comply with the anatomy of the pelvic floor. Therefore, new material and manufacturing-based strategies have to be implemented in order to reduce the risks associated with the use of meshes and to guarantee their integration with the surrounding tissues. In this research work biodegradable, piezoelectric and antibacterial meshes made of polycaprolactone (PCL), polyvinylidene fluoride (PVDF) and levofloxacin (LFX), have been processed via melt-extrusion 3D printing (3DP) aiming to produce a drug-eluting implant with antibacterial activity and with the potential to actively trigger cells thus guiding tissue regeneration.

METHODOLOGY
PCL, PVDF (5%, 10%, 16% w/w) and LFX (0.5% w/w) powders were dry mixed and then used to manufacture honeycomb-shaped meshes. All the raw materials were characterised according to their thermal behaviour, amount of beta phase and their physicochemical properties. The morphology of the produced meshes was analysed via scanning electron mycroscopy (SEM), whereas the mechanical behaviour and meshes storage stability were assessed via stability studies up to 4 months. In vitro tests were also performed (drug release, degradation behaviour, antibacterial potential, cytotoxicity) to assess meshes performances.

RESULTS
Among the tested concentrations the one containing 5% w/w of PVDF proved to be the most appropriate in terms of printability and mechanical behaviour. Thermal analysis showed that no degradation occurred during manufacturing. The amount of beta phase within the different samples did not change significantly after printing and it was around 60% for all samples tested. Meshes were characterised by large pores (1.20 mm), suitable to allow cells colonisation. The evaluated mechanical properties were compared with the native human ones, and found to be close to the physiological range (EPCL/5PVDF/0.5LFX=12.74 ± 0.26). Meshes were capable to release LFX for at least 72 hours, with 60% of the total drug released in three days from PCL/5PVDF/0.5LFX samples.

CONCLUSIONS
The results obtained within this study are promising, as they show how the produced meshes have the potential to satisfy the morphological and mechanical requirements to successfully

manage POP, but also, considering the evaluated release profile, and thanks to their high pre-poling amount of beta phase, the potential to recruit fibroblasts by exerting an antibacterial action and piezoelectric effect.

keywords: 3D Printing, Drug Delivery, Mesh Implants, Pelvic Floor Dysfunctions, Biomaterials

83767245368

3D-PRINTED SHEAR PLATFORM FOR ENDOTHELIAL CELL MECHANOINDUCTION

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Endothelial cells (EC) are subjected to mechanical stimuli and respond via mechanotransduction in vivo. Endothelial cells aligning along the laminar fluid flow direction is widely known to be a morphological feature of vascular function1. Advances in tissue engineering of dynamic vascular tissue models have been increasing day by day1–3. However, the effect of flow on EC pathophysiology has been mostly studied in two-dimensional (2D) in vitro cell culture platforms or microfluidic devices. To fill this gap, we here designed and fabricated a 3D-printed platform that allows a steady laminar fluid shear that is important for healthy vascular tissue dynamics. Moreover, the platform provides controlled laminar flow areas to create complete in-plane intercellular stress fields within the human endothelial cell monolayer on porous substrates. Together with human endothelial cells, this simple and cost-effective platform offers an alternative solution to mimic vascular tissues to identify new therapeutic targets in vascular disease and significantly increase our understanding of the mechanobiology of endothelial cells.

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keywords: 3D printing, mechanotransduction, endothelial tissue engineering

73296328964

A PRE-CLINICAL SHEEP MODEL FOR THE ASSESSMENT OF CRITICAL-SIZED BONE DEFECT RECONSTRUCTION

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Introduction
Market estimates show that the bone replacement market is estimated to reach USD4.94 billion by 2030, within the orthopaedic, oral and maxillofacial reconstructive sectors. Statistics provided by National Center for Biotechnology Information, estimated that in 2015 around 7 million people underwent bone replacement procedures in America. According to the World Health Organization, between 2010 and 2050, the geriatric population in developing and developed countries is projected to increase by 250% and 71%, respectively. Currently, conventional reconstructive techniques for bone defects are challenging, and the limitations associated with donor site availability and morbidities demands clinically translatable alternatives, including scaffold guided bone tissue engineering (SGBTE) approaches. Irrespective of the numerous scaffold designs that have been investigated in the last 30 years, the number of SGBTE approaches reaching clinical application are limited. Amongst the hurdles to meet patient-specific needs, one of the greatest challenges for a SGBTE device to reach the clinic lies with the USA Federal Food and Drug Administration (FDA) regulatory approval. Biocompatibility with human tissues and a history of well-established and standardized pre-clinical models is an essential condition to acquire FDA consent. To thoroughly mimic human in vivo conditions, and to evaluate the effects of SGBTE on critical-sized bone defect regeneration, several large animal models have been developed. Amongst the pre-clinical models published so far, most of them are not well devised, described, and standardized and therefore remain challenging to replicate and to translate into clinical settings. Here we describe our well-established sheep animal model as a pre-clinical tool for evaluating SGBTE.

Methodology
A comprehensive set of procedures to establish a critical-sized bone defect in a sheep model is provided by following four steps: (i) preoperative planning and preparation, (ii) surgical approach, (iii) postoperative management, and (iv) post-mortem analysis.

Results
Several studies have been undertaken using this protocol, including a variety of SGBTE concepts in combination with autologous bone grafts, autologous and allogenic mesenchymal bone marrow precursor, platelet rich plasma, and bone morphogenic proteins. Most studies utilised

medical grade polycaprolactone scaffolds. Replication of this protocol for peer-reviewed publications can be achieved within two year.

Conclusion
Although the use of large animal models has been recommended by the FDA, performing in vivo pre-clinical studies using large animal models, entails high costs and are dependent on a lengthy list of factors prior to full pre-market approval. Since large financial costs are required for advancing clinical trials, pre-clinical research entails narrowing numerous novel treatments and acquiring reliable evidence of a treatment's clinical utility in a proxy species. Sheep are amongst the most suitable large animal model recapitulating human load-bearing conditions, a major requirement for scaffold translation into clinical settings. The use of well-established and clinically-relevant models combined with thorough, reproducible surgical and post-explanation analysis enables us to produce strong scientific data which in turn enables direct comparison between different treatment options for critical-sized defect repair – a shortfall with other less established models. Finally, it offers the ability to improve human predictability, mapping biomaterial's degradation and can address longevity of therapeutic benefit.

keywords: bone tissue engineering, critical-sized bone defects, FDA-approved, large animal models, PCL

94238129528

ASSESSMENT OF SOFT TISSUE COMPONENTS IN A TISSUE ENGINEERED CONSTRUCT: A CRUCIAL STEP TO CLINICAL TRANSLATION

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Harnessing advancements in biodegradable polymers has made tissue engineering an increasingly viable alternative, offering the benefits of established approaches whilst minimising their drawbacks. Whilst the choice of material, indication for use and ideal reconstruction are for debate, a reproducible method of assessment is required to allow for optimisation of pre-clinical technology as well as safely assess in-vivo performance of tissue engineered constructs. A multi-modal method for assessment is presented, incorporating clinical radiographic techniques and histopathological analysis.

Traditional CT and MRI were used both in vivo and on explanted specimens from pigs implanted with a medical grade polycaprolactone scaffold 1 year after implantation. Following sacrifice histomorphometric assessment with haematoxylin and eosin, and Masson's trichrome staining were performed. Immunohistochemistry stains for adipose tissue (Perilipin 1), vasculature (von Willebrand Factor) and macrophage activity (CD68, CD206, iNOS) were also done.
Morphology assessment with CT and MRI was well correlated with histological findings in terms of tissue composition – demonstrating a balance of viable adipose tissue with supporting fibrous tissue adjacent to scaffold architecture. Of particular interest, both radiographic and histological assessment demonstrated no signs of capsule formation. Immunohistochemistry showed a predominance of M1 macrophage activity adjacent to scaffold struts, whilst well-vascularised adipose tissue was demonstrated throughout the analysed specimen.
The ability to correlate cost-effective radiographic methods of analysis with established histological techniques provides an opportunity to both understand the underlying physiology and regenerative pathways as well as clinically monitor tissue growth in a tissue-engineered construct

keywords: Tissue engineering, histology, soft tissue reconstruction

52354566069

CONVERGENCE OF MACHINE VISION AND MELT ELECTROWRITING

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Introduction
Melt electrowriting (MEW) is an additive manufacturing (AM) technology that accurately fabricates polymer fibres onto a collector. The characteristic feature of the technology is a strong electric field between the nozzle and the collector that allows to achieve microscale resolution of the fibre, from 0.8 to 50 microns[1]. With the nozzle raised above the collector, the material deposition process can be accurately monitored using a camera. Machine vision (MV) is a method that enables real-time monitoring and analysis of a process using a camera. Recognizing the successful implementation of MV in metal AM[2,3], here the concept was applied to MEW to analyse the stability of the printing process by measuring three process signatures (Taylor cone area, jet angle, fibre diameter) across three different electric field environments (decreasing, increasing, constant) and control with two air pressure levels (0.4 and 0.7 bar) for each group.

Methodology
A custom-built MEW printer with both flat and tubular collectors was operated to perform printing experiments. Medical-grade poly(ε-caprolactone) was used to fabricate a continuous “U” shaped printing path to capture fibre pulsing with a high-resolution camera (Alpha 7 III, Sony Corporation, Japan) and telescopic lens (ED AF Micro NIKKOR 200 mm lens, Nikon Corporation, Japan). For experiments where the parameters were adjusted during the process, the change in voltage or collector distance was made for each printed layer. An image analysis algorithm developed in MATLAB was used to measure Taylor cone size and the angle of the jet flight from the images captured with the camera. Fibre diameter was measured using SEM (Carl Zeiss Microscopy, Göttingen, Germany).

Results
It was shown that all process signatures reveal information about the stability of the printing process. When the collector distance is increasing during the print, the process signatures commence an oscillatory behaviour due to a decrease in the electric field over time indicating unstable printing conditions. In a constant electric field environment, however, the decreasing electric field is compensated by incrementally increasing high voltage which results in a stable fabrication process. The new knowledge about the stability of the process was translated to the mandrel collector to print 150-layer high-quality tubular scaffolds. Additionally, it was demonstrated that the Taylor cone area provides more accurate and more reliable information about printing stability and fibre diameter during the print when compared to the jet angle.

Conclusion
The Taylor cone volume was identified as an important process signature for process quality identification in MEW. MW was applied to analyse the stability of the printing process in real-time. The investigation allowed to gain a better understanding of the effect of the electric

field on the quality of the printed fibres. The findings were used to manufacture thicker layer constructs. This work demonstrates the importance of real-time monitoring in multiparametric systems such as MEW.

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keywords: 3D printing, melt electrowriting, machine vision, automation, polycaprolactone

83767216005

MECHANICAL PROPERTIES AND PRINTABILITY OF ALGINATE-GELATIN HYDROGELS FOR PRECISE 3D BIOPRINTING

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3D bioprinting is a promising technology to fabricate complex tissue replacements layer by layer through the deposition of cells and biomaterials in a predefined path. It allows us to fabricate complicated geometries that are impossible to obtain through conventional manufacturing methods [1]. Before printing, a computational design for the intended tissue construct needs to be generated together with a corresponding path file. The bioprinter follows the provided printing pattern to create the desired product from the prepared material, e.g., a hydrogel. During the fabrication process, the printability of the hydrogel plays an important role to achieve high accuracy and a good agreement between the printed structure and its computational design. However, it is often challenging to appropriately control the printability of hydrogels. In this study, we have introduced a cooling step and carefully adapted the nozzle temperature to optimize the printability of hydrogels. We have used an alginate-gelatin hydrogel system of 2 % (w/v) alginate - 5% (w/v) gelatin as it provides a cell-friendly environment and is easy to prepare and use. We examined both the mechanical properties and printability of the hydrogel. Due to the long gelation time and the constant change in viscosity as well as printability during fabrication, it was initially impossible to print a well-defined structure precisely. Through a cooling step before the bioprinting process, we induced early thermal gelation and assessed its effect on the hydrogel properties. Moreover, we studied the effect of nozzle temperature on both mechanical properties and printability to identify a suitable temperature for printing. Our results show that the cooling process stabilizes the hydrogel viscosity at different temperatures. In addition, we identified an appropriate cooling time for optimal gel stability. By decreasing the nozzle temperature, we could also decrease the viscosity variation, but it resulted in lower extrudability and uniformity of the gel. In conclusion, the cooling step is beneficial for the bioprinting of gelatin-based hydrogels, and it is key to identify the appropriate temperature to achieve precise printing patterns. Our presented approach will also be applicable for cell printing studies. Moreover, the printability of other concentrations of alginate-gelatin hydrogels could be improved by using the introduced process. Finally, the presented analyses form the basis for computational simulations in the future that will allow us to numerically tune the mechanical properties of the tissue constructs towards those of native tissues [1].

keywords: 3D Bioprinting, Hydrogel, Printability, Mechanical properties

31412731705

MELT ELECTROWRITING OF THIN MEMBRANES, CURVED, AND VARIABLE SECTION TUBULAR SCAFFOLDS

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Melt electrowriting (MEW) has been demonstrated as one of the few biofabrication modalities capable of forming extremely thin micron-sheets for tissue engineering applications (TE). This advantage has been applied to produce planar scaffolds that mimic the tissue in patient-specific anatomical structures e.g., bone and cartilage. Despite the progress in the field, there is a need for further research to manufacture scaffolds with other relevant anatomical shapes. Some of the more complicated anatomies to print out involve curved surfaces and variable cross-section tubes for vascular grafts. Therefore, a methodology is presented here to approach the fabrication for these different geometries. In order to ensure the accuracy of fiber deposition for planar and tubular scaffolds, we have achieved optimal control over process parameters such as voltage, pressure, and rotation/translation speed. Also, the relevant role of the programming of kinematics is showcased in our MEW device with which we have obtained scaffolds of stacked micro-fibers with pore sizes of approximately 500 μm. Work is still ongoing to develop more generic solutions and methods that further expand the range of geometries that can be produced. Nevertheless, the proof-of-concept presented here represents a significant progress in the manufacturing of scaffolds with anatomically relevant shapes.

keywords: melt electrowriting, scaffolds, polycaprolactone, additive manufacturing, biomedical devices

41883636547

PERFUSION DEVICE FOR 3D GASTROINTESTINAL SPHEROIDS

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2D (two-dimensional) monolayers of Caco-2 cells, on Transwell inserts, have been widely employed to assess drug permeability and absorption of drugs across the intestine. However, 3D (three-dimensional) in vitro tissue models are more physiological relevant as they can simulate interactions with the extracellular matrix and, therefore, better recapitulate the complex in vivo intestinal tissue environment [1–5]. Thus, the characteristics of these Caco-2 spheroids, including their barrier function, potential changes in cell phenotype and transport function, need to be well-understood. With this motivation, we designed a 3D-printed device to culture geometrically controlled Caco-2 spheroids. Furthermore, we developed the device to enable the perfusion of medium to overcome the limitation in cell-construct size due to mass exchange. We conduct a comparative study to evaluate static and dynamic conditions to validate the advantage of spheroid culture with perfusion. This alternative in vitro intestinal model will serve as a valuable system to expedite drug screening as well as to study intestinal transporter function.

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keywords: 3D printing, perfusion, lab-on-a-chip, gastrointestinal tissue engineering

62825439644

RHEOLOGICAL CHARACTERIZATION AND COMPARISON OF PRINTING HYDROGEL-BASED COMPOSITE INKS FOR EXTRUSION-BASED 3D PRINTING

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3D printing, or spatial printing, is a modern, low-cost alternative to earlier manufacturing technologies. It involves printing layer upon layer of an object designed in an appropriate digital file. As inks in 3D printers are used various filaments, most often polymeric, in solid form, which are melted in the printer head. However, the medical industry is increasingly using hydrogels as inks for 3D printers. Such inks, depending on the application, should exhibit appropriate properties, such as viscosity or pH [1]. 3D printed models based on hydrogels are used in tissue engineering and regenerative medicine (e.g. as scaffolds that support healing). One of the biggest problems with 3D hydrogel printing is creating the right kind of gel that will create the right environment for cell growth, while having the right physicochemical properties to accurately reproduce the 3D model of the tissue [2,3]. Due to the requirements that must be fulfilled by the hydrogel-based ink, new and improved biomaterials are constantly being sought to provide the possibility of obtaining the expected models with specific applications.

The aim of this study was to select hydrogel composite materials suitable for 3D printing by extrusion. The composite mass consisted of a natural polymer based hydrogel enriched with osteogenic particles. Studies included rheological characterization, pH testing, and printability testing of the hydrogel inks.

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keywords: hydrogels, composites, rheology, 3D printing

31412749917

SCAFFOLD GUIDED TISSUE ENGINEERING FOR THE TREATMENT OF ABDOMINAL WALL AND PELVIC ORGAN PROLAPSE - SHEEP MODEL

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Introduction. The long-term expected outcome after a hernia mesh repair is to create a framework in the prolapsed area that facilitates the development of mechanically robust, vascularized new tissue formations. Hernias of the anterior abdominal wall and vaginal prolapse are common and traditional suture repairs are associated with high rates of recurrence. Similarly, permanent meshes, while effective, often lead to significant patient morbidity in long term. For example, in Australia, transvaginal meshes were introduced in 2004 and unfortunately, while the meshes were successful in treating the prolapse, the material led to several serious complications, including pain and erosion into surrounding organs. Since 2017, most of these products have been removed from the market with worldwide litigation ongoing. To address the limitations of conventional permanent meshes, we developed 3D-printed biodegradable meshes with biomimetic mechanical properties and hypothesized that our medical-grade polycaprolactone (mPCL) scaffolds loaded with platelet-rich plasma (PRP), will enhance soft tissue regeneration in fascial defects in abdominal and vaginal sheep models.

Methodology. A pre-clinical evaluation of novel mPCL mesh scaffold concept for the vaginal and abdominal hernia reconstruction was undertaken along with the control of clinically-applied polypropylene (PP) meshes using an ovine model. Each sheep was implanted with both a PP mesh and a mPCL mesh loaded with PRP in both abdominal and vaginal sites for six months. Mechanical properties of the explanted tissue-mesh complexes were assessed with plunger tests. Tissue responses to the implanted meshes were evaluated via histology, immunohistochemistry and histomorphometry. Native fascial samples were also harvested from the animals for negative control comparison.

Results. The preliminary results obtained fromthis six month-long pilot study suggest that the surgical implantation of both grafts is safe. We could not find any signs of wound infection or graft erosion into the surrounding viscera. The explanted mPCL mesh/tissue complexes were measured to be less stiff than the PP mesh/tissue complexes, but stiffer than the native tissue, while showing equitable collagen and vascular ingrowth when compared to PP mesh/tissue complexes.

Conclusion. Our initial findings indicate that biodegradable mPCL scaffolds are safe and effective meshes for treating hernia and vaginal prolapse in short term (6 months), hence a full-scale long-term study (over 24-36 month) with an adequate sample size is recommended.

keywords: PCL, tissue engineering, large animal models, hernia

PS34
Advanced therapy approaches in tissue engineering

73296360939

INNERVATION IN BONE TISSUE HEALED WITH CHEMICALLY MODIFIED RNA

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Introduction
The formation and outgrowth of nerve fibers during fracture healing is a highly important process. These nerve fibers are distributed throughout the entirety of skeletal tissue, being crucial for sensing and responding to stimuli such as mechanical load. Bone morphogenic proteins (BMPs) are commonly used for bone tissue regeneration, due to their osteogenic character. However, this growth factor family does not induce the formation of nerves.
Thus, identifying nerve growth cues, intrinsically expressed during bone healing may be advantageous. The clinical use of recombinant protein poses several limitations. These include, but are not limited to, adverse side effects in patients due to the unavoidable use of supraphysiological amounts along with high costs. Gene- and transcript-therapies are proposed as alternatives to recombinant protein use. Transcript therapy is based on the application of protein-coding messenger RNA (mRNA) to induce in situ protein production by the local cells.
As mRNA is unstable and immunogenic, chemical modifications to mRNA (cmRNA) increase its stability and biocompatibility. BMP-2 cmRNA has been proven to induce osteogenesis in vitro and in vivo1-3.
In this study, expression of semaphorin-3A (SEMA-3A), neurofilament 200 (NF-200), and b3-tubulin (B3T) in rat BMP2 cmRNA-treated bone specimens was evaluated. These molecules are crucial in neuron development.

Methodology
In rats, 5 mm femoral defects were treated with a collagen sponge containing 5-50µg BMP-2 cmRNA. As a comparison, 11µg of recombinant human (rh) BMP-2 was used. Bone tissue explants were harvested at 4- and 8- weeks after implantation. Expression of SEMA-3A, NF-200, and B3T was assayed by qPCR and immunohistochemistry (IHC). For qPCR, explants were homogenized in TRIzol using a TissueLyser (Qiagen, Germany; 3 min at 30 Hz). RNA extraction and subsequent cDNA synthesis were performed using standard protocols and kits3. Human β-tubulin was used as the housekeeping gene. The ΔΔCt method was used to treat the data. For IHC, explants were fixated, decalcified, and embedded in paraffin. Staining was performed using DAPI and an anti-SEMA-3A, anti-NF-200, or anti-B3T antibody (Abcam, UK). Samples were imaged using a Slide Scanner microscope (Nikon, Japan) for fluorescence microscopy.

Results and conclusion
Healing doses of BMP-2 cmRNA (25µg and 50µg) showed the highest expression of SEMA-3A, NF-200 and B3T. SEMA-3A, NF-200 and B3T expression was particularly observed in callus tissue and de novo formed bone. The expression of SEMA-3A was less specific than that of NF-200 and B3T, as SEMA-3A has several functions other than neurogenesis. This expression was observed as early as 4 weeks after cmRNA treatment and persisted up to 8 weeks. Similar expression patterns were observed when using the rhBMP-2 counterpart. Conversely, untreated defects showed little to no expression of these genes.

Funding
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keywords: Transcript therapy, bone engineering, innervation, cmRNA

20941838087

NON-VIRAL GENE THERAPY FOR RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA: HYPER BRANCHED AMINATED POLYESTERS MEDIATED MINICIRCLE DNA DELIVERY

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Introduction
Recessive dystrophic epidermolysis bullosa (RDEB) is a rare autosomal inherited skin blistering disorder caused by mutations in the type VII collagen gene (COL7A1). The large size of COL7A1 is a big delivery challenge for both viral and non-viral vectors and seriously hindered the development of therapeutic gene replacement therapy for RDEB. Our group has developed a series of hyper branched aminated polyesters (HAPE) as high-performance gene delivery vectors and combined them with a minicircle DNA coding full COL7A1 gene as a non-viral gene replacement strategy for therapeutic treatment of RDEB [1-2]. Here we further synthesized new HAPE with greater DNA delivery ability, higher gene expression efficiency and better biocompatibility.

Methodology
A new HAPE family was developed via an “A2+B3+C2” Michael addition strategy and were evaluated using green fluorescent protein (GFP) coding plasmid on human embryonic kidney 293 (HEK) cells and human recessive dystrophic epidermolysis bullosa keratinocyte (RDEBK) cells. The selected HAPE was further optimised with molecular weight and branch ratios for the best performance on DNA transfection on RDEBK cells. Then the optimised HAPE was formulated with minicircle DNA coding full human COL7A1 gene (MCC7) into nanoparticles, and its therapeutic effect was confirmed on RDEBK cells by quantitative polymerase chain reaction, immunocytochemistry staining and immunoblotting on both transcription and translation levels.
Results: The best HAPE (HAPE-B) had around 3 times higher GFP expression levels than other developed HAPEs on HEK cells. MCC7 delivered by further optimized HAPE-B (HAPE-BO) showed more efficient restoration of type VII collagen than the commercial reagents, Lipofectamine 3000 and JetPEI, on RDEBK cells.

Conclusion
The newly developed HAPE-BO can efficiently deliver the full COL7A1 gene into RDEBK cells in a cytocompatible manner and successfully restore the functional type VII collagen expression. The improved system developed here has a high potential for use as an efficient and safe non-viral topical treatment for RDEB patients in the clinic and can be adapted to other genetic diseases.

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keywords: recessive dystrophic epidermolysis bullosa; gene replacement strategy; hyper branched aminated polyesters; non-viral vector; minicircle DNA

PS35
Giving meaning to early tissue damage responses in regeneration

20941837244

HARNESSING THE ADVANTAGES OF EX VIVO MATERIALS TO EXPLORE IMMEDIATE WOUNDING RESPONSES OF THE SKIN

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Wounding affects us all, yet the mechanisms of healing are still a partial mystery. In particular the events immediately unfolding when a tissue is wounded are difficult to capture, and are thus understudied und incompletely understood. Studying wounding responses in ex vivo skin is an elegant strategy to explore immediate wounding responses. Using ex vivo skin from both human and pig has allowed us to unravel some of the pathways triggered when skin is damaged through induction of an excision wound. We have found that ex vivo skin is able to mount a fast and strong response to wounding, and this response is upheld for at least six hours after wounding. This response can be manipulated with drugs, labeled with proliferation and protein translation trackers and quantified through histological analysis. The use of ex vivo skin opens the doors to complex experiments not possible in living organisms: adding and removing several drugs on separate pieces of skin in parallel, or observing tissue responses in anaerobic wound conditions. Moreover, the use of human ex vivo skin has allowed us to replicate excision wounds in humans, by utilising waste material from surgical operations. Our methods have allowed us to identify novel damage-induced tissue responses resulting from skin injury.

keywords: skin, wound healing, ex vivo, immediatell.

73296334266

UTILIZING MICROBE-DERIVED AGENTS TO MODULATE INFLAMMATION AND SKEW OSTEOGENESIS IN CERAMIC-BASED BONE SUBSTITUTES

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Introduction
Bone grafting is among the most frequent forms of transplantation, utilized to manage a variety of bone defect cases. Enriching synthetic bone substitutes with immune-interacting properties can be a strategy to improve their performance. The host's response to e.g. microbial components can result in both a positive or negative effect on bone formation. As an immunomodulatory strategy, we tested a variety of microbial-derived agents on their potential to enhance osteogenesis in biphasic calcium phosphate (BCP) scaffolds.

Materials and Methods
Implant preparation
BCP discs (6x9mm ø) comprising 65%-75% tricalcium phosphate and 25%-35% hydroxyapatite with a porosity of ±75% were used. Microbes tested included inactivated Staphylococcus aureus, Candida albicans, and Bacillus Calmette–Guérin (BCG) in a concentration of 105 -107units/ml. Microbial components tested included peptidoglycan, poly (I:C) high molecular weight, CpG oligodeoxynucleotid type C, Pam3CSK4, and curdlan at a concentration of 0.1μg/ml (low) and 1-10μg/ml (high). Microbial agents were diluted in phosphate buffered saline and combined with bone morphogenetic protein-2 (BMP-2, 5μg/scaffold), subsequently 200μl of the solution was loaded onto the scaffolds (n=10-12).

Animal Study
24 New Zealand White Rabbits (female, 4.5-5 kg) were included under the approval from the local Committee for Animal Experimentation. To pre-test the inflammatory response towards microbial agents, peripheral blood was collected, stimulated, and analyzed for the presence of IL-6 and IL-1β by ELISA. Scaffolds were implanted in subcutaneous pockets of the rabbits, with a control group (BCP with BMP-2) present in each animal. On week 2 and 3, fluorochrome labels calcein green and xylenol orange were administered. Fluctuations in body weight and temperature were monitored weekly. After a 5-week implantation period, samples were analyzed by histomorphometry (basic fuchsin/methylene blue) and fluorochrome imaging.

Results
Microbial agents stimulated a dose-dependent response in rabbit blood with increased levels of both IL-6 and IL-1β. As expected, the suboptimal dose of BMP-2 triggered moderate osteogenesis. Bone was present in only 17 of 23 rabbits, and a relatively high variation of bone

area percentage was seen in the control group: 5%(7.9) Most of the microbial components tested showed an inhibiting trend towards bone formation, except for BCG and peptidoglycan. The mean differences (SD) in bone area percentage compared to the control were as follows; Staphylococcus aureus low -5%(8.1) and high -2.2%(6.1); Candida albicans low -3.2%(7.8) and high -4.1%(8.2); BCG low -0.7%(5.0) and high 0.3%(5.9); peptidoglycan low 2.3%(4.8) and high 3.9%(11.9); Poly(I:C) low -6.8%(9.6) and high -2.8%(7.4); CpG C low -2.8%(9.9) and high -0.9%(7.4); Pam3CSK4 low -2.1%(3.8) and high -0.4%(4.5); and curdlan low -2.7%(4.4) and high -2.7%(4.4). Interestingly for several groups, lower concentrations showed a stronger trend of hindering osteogenesis than higher concentrations.

Conclusion
The microbial agents can evoke an inflammatory response in rabbit blood. This response did not directly translate to effects on bone regeneration when such agents were loaded on BCP in the presence of suboptimal BMP-2 dosages. Further investigation is needed to determine the optimum range of concentrations in which microbial agents may have a beneficial effect on bone formation.

keywords: Osteoimmunology, immunomodulation, calcium phosphate, in vivo

PS36
Glycomodulation Approaches in Tissue Engineering

52354546155

INFLAMMATION-INDUCED CHANGES IN THE GLYCOSYLATION AND METABOLISM OF HUMAN CORNEAL FIBROBLAST ARE AMELIORATED BY A CHEMICAL INHIBITOR OF FUCOSYLATION

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Introduction:
Dysregulation of glycosylation and metabolism are associated with pathological inflammation across various biological tissues1. Research into this dysregulation in the cornea has indicated that inflammation can induce changes in the structure and composition of corneal glycans2. An improved understanding of these alterations and the metabolic pathways leading to glycan synthesis could help identify novel diagnostic factors and therapies for corneal inflammation. Herein, we analyzed changes in glycosylation in healthy and inflamed primary human corneal fibroblasts (HCFs) by lectin immunohistochemistry and metabolism by a Seahorse XF Mini Analyzer. We then assessed the capacity of a glycosylation inhibitor to prevent cytokine-induced hyperfucosylation and ameliorate mitochondrial dysfunction.

Methodology:
We developed an optimized protocol to induce inflammation in primary HCFs with 20 ng/mL of IL-1β or TNF-α for 72 hours, with or without 300 μM 2F-Peracetyl-Fucose (2F-PAF), a fluorinated inhibitor of fucosylation. After treatment, cells were fixed and stained with eight glycan-binding, fluorescein-tagged lectin proteins. These lectins bind to glycans with mannose (ConA, GNA), fucose (AAL, UEA I), sialic acid (SNA), N-Acetylgalactosamine (WFA, PNA) or N-Acetylglucosamine (WGA) residues. Cytokine with/without 2F-PAF treatments were given to the cells for Seahorse metabolic analysis. Seahorse XF Cell Mito Stress Tests were carried out on healthy cytokine with/without 2F-PAF-treated HCFs, by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) to analyze mitochondrial health.

Results:
Our results demonstrated that for healthy cells, the only lectins with robust fluorescence were the α-linked mannose-binding ConA and GNA lectins, with very weak fluorescence for the other lectins in the panel. In the cytokine-stimulated HCFs however, there were increases in WGA and PNA fluorescence, along with statistically significant increases (2.28- and 2.68-fold increases for IL-1β and TNF-α, n = 3) in AAL fluorescence, which binds to α1,3 or α1,6 linked fucose. Changes for UEA I (α1,2 linked, terminal fucose) and SNA (α2,6 sialic acid) were not significant. 2F-PAF treatment inhibited cytokine-induced core fucosylation in the HCFs (77.49% and 75.96% decreases for IL-1β and TNF-α, n = 3) and did not affect terminal fucosylation. In Seahorse XF Cell Mito Stress Tests, the basal and maximal respiration rates measured in OCR, increased (1.74- and 1.57- fold respectively, n = 3) in IL-1β-treated HCFs relative to healthy cells. In cells stimulated by IL-1β with 2F-PAF, basal, and maximal respiration reverted to healthy cell levels.

Conclusions:
In corneal inflammation, increased proinflammatory cytokines are associated with altered glycosylation. The data from our cytokine assays showed similar effects, with cytokine stimulation inducing hyperfucosylation in HCFs. 2F-PAF, a fucosylation inhibitor, prevented this hyperfucosylation. Inflammation caused mitochondrial dysfunction in the cells, which 2F-PAF

could also ameliorate. Together, these data indicate that inflammation induces hyperfucosylation and mitochondrial dysfunction in corneal fibroblasts and that 2F-PAF has therapeutic potential in corneal inflammation. This work lays the foundation for a biomaterial-encapsulated fucosylation inhibitor treatment for corneal inflammation.

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keywords: Glycosylation, Metabolism, Cornea, Inflammation, Fucosylation

PS37
Human Organoids for Musculoskeletal Tissues

41883637084

TOMOGRAPHIC VOLUMETRIC PHOTOFABRICATION OF LIVING IN VITRO BONE MODELS

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INTRODUCTION
Light-assisted additive bio-manufacturing has emerged as a powerful tool for custom fabrication of structurally complex 3D tissue models from stem cells.[1-2] Existing in vitro bone models, however, often lack terminally differentiated bone cells named osteocytes which are crucial for bone homeostasis and functional adaptation in response to mechanical loads. Here, we report ultrafast tomographic photofabrication of centimeter-scale heterocellular bone constructs that enabled osteocytic differentiation of human mesenchymal stem cells (hMSCs) within hydrogels after 42 days of 3D endothelial co-culture.[3]

METHODS
A series of bioresins with varying amounts of gelatin methacryloyl (GelMA) and photoinitiator lithium phenyl-2,4,6- trimethylbenzoylphosphinate (LAP) were formulated and printed on a Readily3D Tomolite bioprinter. Eosin Y staining and confocal imaging was used to assess the printing precision. Photo-rheology was applied to assess printability and gel mechanics. Cytocompatibility was evaluated by live-dead and actin-nuclei staining after 3D osteogenic culture for 7 days. Cell activity in 3D mono-culture and co-culture with human umbilical vein endothelial cells (HUVECs) was compared by weekly ALP and qPCR assays for up to 42 days.

RESULTS & DISCUSSION
To create a permissive environment for embedded cells, we screened a series of bioresin formulations in terms of photo-reactivity, gel mechanics, cell-compatibility, and the ability to support osteogenic differentiation. A variety of CAD models (femur, branched vasculature) can be printed by tomographic photofabrication within 30 seconds. The laser dose is dependent on LAP and GelMA concentrations. We identified an optimal bioresin comprising 5% GelMA, 0.03% LAP and 2 M/mL hMSCs. After printing cells were highly viable (>90%), indicating good biocompatibility of the resin and the printing process. However, increasing the LAP concentration led to a decrease in cell spreading. After 7 days cells showed long dendritic processes. Compared to a bioresin with 10% GelMA,[2] our resin led to an upregulated expression of osteogenic gene markers. After 42 days, a significantly increased gene expression of both osteoblastic markers (collagen-I, ALP, osteocalcin) and osteocytic markers (Podoplanin; Dmp1) was evidenced in the 3D co-culture. The gel compressive moduli increased from 6 kPa to 46 kPa, indicating the increased matrix secretion. Finally, we developed a pre-vascularized model by injecting HUVECs into a printed gel construct containing hMSCs. After 2 days HUVECs self-organized into an endothelium lumen, forming a pre-vascularized tissue construct.

CONCLUSIONS
Using an optimized bioresin, this study leverages the benefits of ultrafast tomographic bioprinting and 3D co-culture for scaled additive photofabrication of 3D living bone tissues with an in vivo-like heterocellular environments and long-term functionality.

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keywords: Musculoskeletal, bone, organoids, volumetric bioprinting

PS38
Injectable biomaterials for cell-instructive matrix cues

41883607147

SPATIOTEMPORALLY INSTRUCTING ENGINEERED LIVING MODULAR TISSUES VIA BIOCHEMICALLY AND BIOPHYSICALLY TUNABLE MICROBUILDING BLOCKS

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Introduction:
Modular tissue engineering exploits the 3D self-assembly of cells and building blocks to create larger tissue constructs with higher complexity and resolution.[1] However, current modular tissue engineering strategies have near-exclusively relied on static, non-responsive, micromaterials,[2] whereas functionality of native tissues is dictated by their inherently dynamic nature.[3] Here, we report on smart, dynamically tunable, microbuilding blocks of which the biochemical and biophysical properties can be altered via well-controlled secondary crosslinking strategies. The mechanical properties of the building blocks were modified post-synthesis by exploiting visible-light-induced secondary crosslinking of the free tyramines using ruthenium (Ru) and sodium persulfate (SPS) as initiators. Free biotins could be stepwise functionalized with biotinylated molecules of interest using competitive supramolecular complexation of avidin and biotin analogs.[4] The spatiotemporal control over mechanical and chemical properties of smart building blocks within living modular tissues provided a highly tunable, well-defined, and dynamic cellular microenvironment, which allowed for the in situ modification of (stem) cell behavior and fate.

Methodology:
Microbuilding block production and functionalization: Hydrogel precursor droplets composed of 5% (w/v) Dextran-Tyramine-Biotin (DexTAB; ~1 mM biotin) and 22 U/mL horseradish peroxidase in phosphate buffered saline (PBS) were emulsified in 2% (w/w) Pico-Surf 1 containing Novec 7500 Engineered Fluid using a microfluidic droplet generator with subsequent crosslinking on a separate controlled hydrogen peroxide (H_2O_2) supplementation.[5, 6]

Modular tissue engineering: RGD-functionalized microbuilding blocks were homogeneously co-seeded with cells into non-adherent 3% (w/v) agarose microwell chips at a density of ~50 units per microwell.

In situ stiffening: Building blocks within modular tissues were in situ stiffened by incubating them with 2.5 mM of SPS and 1 mM of Ru. Free radical crosslinking was induced using 60 seconds of visible light irradiation. Lineage commitment was visualized using histochemical staining, and imaged using confocal (fluorescence) microscopy, and analyzed using image analysis software.

Results:
Stepwise functionalization of the microbuilding blocks with c(RGD)fk initiated the self-assembly of cells and microbuilding blocks via integrin-mediated interactions. The cell-matrix interactions

within cell-building block aggregates were optimized to steer stem cell fate towards stiffness-induced osteogenic interactions.

Furthermore, microbuilding blocks were tuned biophysically and biochemically in situ by secondary orthogonal crosslinking schemes. A visible-light-induced crosslinking, using Ru and SPS as initiators, allowed for on-demand control of building block stiffness. Stem cell fate toward osteogenic and adipogenic lineages was temporally steered by in situ tuning the building block stiffness within modular tissues. In situ biochemical control over cell-building block aggregates was shown by temporally endowing the building blocks with (desthio)biotinylated bone morphogenetic protein (BMP)7 neutralizing antibodies, which showed temporal control over the cellular response of a BMP7 reporter cell line.

Conclusion:
In conclusion, we developed the first biochemically, biophysically, and spatiotemporally controlled smart building blocks for modular tissue engineering. This allowed for the creation of highly tunable and defined cellular microenvironments, which more accurately resembled the dynamic microenvironment of cells in native tissues.

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keywords: micromaterials, self-assembly, bottom-up tissue engineering, smart materials;

PS39
Injectable composite hydrogels as scaffolds and drug delivery systems for tissue engineering

94238104205

3D PRINTED STEP-GRADIENT COMPOSITE HYDROGELS FOR DIRECTED MIGRATION AND OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW-DERIVED MESNCHMAL STEM CELLS

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Introduction
In bone tissue engineering, several attempts have been made to generate artificial bone constructs to replace the autograft and allograft treatment and to enhance bone repair and regeneration. However, engineering bone tissue presents some challenges, mainly based on bone tissue's gradual variation of several biological, mechanical, and structural features. Another challenge is to control the migration of stem cells, which have an inherent ability to migrate. Uncontrolled migration of stem cells can cause to pathological situations such as cancer and inflammatory diseases. Therefore, besides promoting stem cell differentiation, it is crucial to regulate stem cell migration in order to achieve their maximum capacity for differentiation and regeneration.

Methodology
We describe mechanically, biochemically, and topographically graded 3D (nano)composite scaffolds (GradS) generated by a 3D printing technique. We prepared our (nano)composite hydrogels by mixing gelatin methacryloyl (GelMA), alginate, and pH-responsive drug delivery nanomaterials [DexPMO-PDL=dexamethasone (Dex)- and poly-d-lysine (PDL)-functionalized periodic mesoporous organosilicas (PMO)] in different ratios. We prepared three different composite hydrogels increasing GelMA and DexPMO-PDL concentration (GA5, GA10, and GA10-P). GA5 was prepared by mixing GelMA (5% w/v) and Alg (7% w/v), while GA10 was composed of GelMA (10% w/v) and Alg (7% w/v). In order to prepare GA10-P, DexPMO-PDL (0.2% w/v) was mixed with GA10. The prepared (nano)composite hydrogels were used for fabricating a new 3D step-gradient (nano)composite scaffold (GradS) made by 3D bioprinting.

Results
Our results showed that the Dex release from the GA10-P scaffold at pH 7.4 and 6.0 was prolonged and pH dependent. After 7 days of incubation, the amount of Dex released from GA10-P scaffold at pH 6.0 was 2 times higher than at pH 7.4, demonstrating GA10-P's ability to perform pH responsive drug delivery. Cell experiments were performed on GradS. we only seeded hBMMSC into the GA5 section of the GradS. Fluorescence microscopy images and cell viability kits showed that hBM MSC migrated from the GA5 part toward GA10-P part of the GradS. Since GA10 and GA10-P have higher GelMa and DexPMO-PDL concentrations than GA5, this migration depended on an increase in the GelMA concentration and on the incorporation of DexPMO-PDL into the hydrogel network.

We also investigated the impact of GA5, GA10, GA10-P, and GradS on hBM MSC differentiation toward an osteogenic lineage. Osteogenic differentiation capacity was determined by alizarin red and BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium) colorimetric staining. We observed a proportional increase in the alizarin red- and BCIP/NBT-stained areas with an increase in the GelMA and DexPMO-PDL concentration. This indicates that the osteogenic differentiation capacity of hBM MSC was promoted by an increase in the content of GelMA and DexPMOPDL within the hydrogel network.

Conclusion
The cell experiments demonstrated that the viable number of hBM MSC in the GradS increased as the content of GelMA and DexPMO-PDL increased. This effect was used to control the migration and osteogenic differentiation of hBM MSC toward the GradS section possessing a higher concentration of GelMA and DexPMO-PDL.

Acknowledgements
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keywords: Gradient scaffolds, 3D-bioprinting, nanocomposite hydrogels

31412714155

A STUDY ON HBM-MSCS CHONDROGENIC COMMITMENT BY 3D COLLAGEN SCAFFOLD LOADED WITH PLGA NANO-CARRIERS FOR TGF-B1 CONTROLLED RELEASE

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Introduction
Treatment of hyaline cartilage lesions remains a major challenge owing to the avascular nature of itself, which prevents spontaneous healing. To date the use of Mesenchymal Stem Cells (MSC) and tissue engineering protocols represents an alternative strategy and offers great potential to improve joint therapy. The development of injectable 3D scaffold able to stimulate the formation of a new cartilaginous tissue is crucial to the success of the therapeutic strategy. Despite collagen-based hydrogel lack of the appropriate mechanical resistance it has been described as excellent matrix to support the chondrocyte and stem cells growth because are more like natural ECM [1,4].

Methods
A 3D collagen scaffold was assembled as biomimetic extracellular matrix for human Bone Marrow Mesenchymal Stem Cells (hBM-MSC) induction towards chondrogenic phenotype by dispersing poly-lactic-co-glycolic acid (PLGA) nano-carriers, carrying and delivering Transforming Growth Factor (hTGF- b1) payload, within the 3D structure. Cultures were performed both in static than dynamic condition (perfusion).

Results
hBM-MSCs early commitment towards a chondrogenic phenotype was confirmed both via immuno-histology that revealed the type II collagen fiber deposition within the scaffold and by q-RT-PCR that indicated the increase of gene expression of SOX9 and COL2A1, after 16 days of culture. The dynamic culture by perfusion assured a better mass exchange and more effective scaffold matrix reshaping.

Discussion and Conclusions
The data suggested the effectiveness of 3D collagen scaffold carrying PLGA microspheres for drug delivery as injectable scaffold and opened perspectives for its use as advanced therapy device to promote cartilage regeneration.

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keywords: PLGA nanocarriers, growth factor delivery, stem cells, collagen hydrogel, chondrogenic commitment, perfusion system

62825439084

COMBINED BORON COMPOUND AND FIBRONECTIN SYSTEM AS A POTENTIAL APPROACH TO THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY.

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This work is based on the functional coupling of integrins, boron (B) transporter (NaBC1), and growth factor receptors (GFR) after their activation. In our previous work, we have demonstrated that active-NaBC1, co-localise with fibronectin-binding integrins ($\alpha5\beta1/\alpha v\beta3$) and GFR producing a functional cluster that synergistically enhances biochemical signals and crosstalk mechanisms, accelerating local muscle repair in vivo after an injury. Based on the data reporting that increased fibronectin (FN) levels are used as a marker of disease in several muscular dystrophies, here we have studied a combined system composed of boron (for NaBC1 stimulation) and FN (for FN-binding integrin stimulation), and evaluated the effects in restoration of dystrophic phenotypes in in vitro and in vivo models of Duchenne Muscular Dystrophy (DMD).

We have used borax (Sodium tetraborate decahydrate) and bortezomib (a dipeptidyl boronic acid) as two different boron compounds. Bortezomib is an FDA approved drug usually employed for the treatment of hematologic malignancies. Human DMD cells have been cultured with soluble borax or bortezomib, alone or combined with FN. Results show that both, borax and bortezomib, combined with FN improved myotube differentiation and diameter. Considering that dystrophic DMD cells present hallmark features such as reduced fusion capability and smaller and thinner myotubes compared to healthy controls, we concluded that both, borax and bortezomib, together with FN, are restoring myotube fusion capability and myotube physiological function (myotube diameter). Interestingly, although the results obtained with both, borax and bortezomib were similar, the borax doses used were in the range of milimolar while the bortezomib doses were in the range of nanomolar (an ultra-low dose compared to its original therapeutic indication), indicating that we obtained similar effects in myotube restoration using ultra-low doses of a boron compound.

After that, we have investigated the effects of ultra-low doses of bortezomib in a DMD mouse model in vivo. We have engineered and characterised injectable PEG-based hydrogels simultaneously presenting cell adhesion peptide domains (RGD) and controlled local bortezomib-release. The selected compositions were injected in both tibialis anterior (TA) of 8 weeks old D2.B10 (DBA/2-congenic) Dmdmdx male mice. Five experimental groups have been evaluated: i) Wild type control mice, ii) DMD control mice, iii) DMD PEG-RGD, iv) DMD PEG-bortezomib and v) DMD PEG-RGD-bortezomib (n = 6). We monitored the evolution of the body weight and motor impairment in behavioural four limb hanging test over 8 weeks. After euthanasia at 12 weeks old, tissues were analysed by histological and immunofluorescence techniques.

Hematoxylin-eosin-stained sections of PEG-RGD-bortezomib treated group, although still displaying typical DMD histopathology, showed a significant decrease in size variability among muscle fibers, mononuclear cell infiltrates, degenerating fibres, and centrally located nuclei. Immunofluorescence analysis revealed that treatment with PEG-RGD-bortezomib significantly reduced FN levels, suggesting a partial prevention of disease progression characterised by massive increase in FN levels.

Altogether, our findings in this local proof of concept suggest that active-NaBC1 is capable, at least partially, of rescuing dystrophic phenotypes in mdx model, opening to new possibilities to develop therapeutic approaches for the treatment of DMD.

keywords: Boron, fibronectin, DMD

31412720286

DEVELOPMENT OF AN INJECTABLE THERMOSENSITIVE HYDROGEL BASED ON CHITOSAN TO DELIVER FUCOIDAN AND ADULT STEM CELLS FOR BONE REPAIR

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Introduction: Hydrogels are promising biomaterials for regenerative medicine due to their flexible shape, resemblance with the extracellular matrix and capacity to deliver and release bioactive compounds. Especially, injectable hydrogels gained popularity due to their minimal invasive application [1]. Fucoidan is a sulfated polysaccharide from the cell wall of brown algae with multiple bioactivities on osteogenesis, angiogenesis and inflammation [2, 3]. In this present study, we developed an injectable thermosensitive hydrogel to deliver fucoidan or cells in the context of bone repair. Biocompatibility and functionality were tested using human bone mesenchymal stem cells (MSC) and outgrowth endothelial cells (OEC). Both cell types represent suitable model systems to evaluate bone regeneration and vascularization [4].

Methodology: The thermosensitive injectable hydrogel was prepared using chitosan, collagen type I and β-glycerophosphate (β-GP). Chitosan in combination with β-GP forms a thermosensitive sol which gelates upon temperature increase [5]. Collagen type I was integrated into the hydrogel formulation to mimic the bone tissue environment and to enhance biocompatibility. Different concentrations of commercially available fucoidan (F. vesiculosus, Sigma-Aldrich) or enzymatically-extracted fucoidan from F. evanescens [6] were encapsulated into the hydrogel. Basic properties such as gelation time, pH and the equilibrium swelling ratio (ESR) were determined. Further, the internal structure of the hydrogels was analyzed by scanning electron microscopy (SEM) after critical point drying. Cultures of human MSC and OEC either seeded on top of the gel (2D) or integrated into the gel (3D) were subjected to life staining and SEM to study the biocompatibility of the hydrogels.

Results: The hydrogels with encapsulated fucoidan gelated within 5 min at 37°C. Fucoidan encapsulation had only minor effects on the studied hydrogel properties. However, the encapsulation of high fucoidan amounts (500 µg/ml) caused a turbid sol and decreased the ESR. Additional experiments revealed that within two days 60 % and after six days 80 % of the fucoidan was released from the hydrogel. Life/dead stainings showed that MSC cultured on top and inside the hydrogels were vital, able to adhere and to spread after six days. OEC cultured on hydrogels with encapsulated enzymatically-extracted fucoidan appeared vital after six days and formed primitive prevascular structures.

Conclusion: This study documents the general applicability to encapsulate fucoidan into the described thermosensitive and injectable hydrogel with a release profile tested up to six days. The biocompatibility for MSC and the observed pro-angiogenic effect by fucoidan integrated into the gel are promising for future applications in bone regeneration.

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keywords: Hydrogel, Fucoidan, Injectable, Bone regeneration

31412737204

DEVELOPMENT OF DECELLULARIZED BONE EXTRACELLULAR MATRIX HYDROGELS FOR REGENERATION OF BONE TISSUE

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There is still need for development of biomimetic hydrogels capable to recapitulate the native environment of bone tissue, as those used currently lack of tissue-specific composition and yield suboptimal tissue formation. Decellularized extracellular matrix (dECM) is composed of tissue-specific biopolymers and growth factors. Moreover, it undergoes gelation at physiological temperatures. Therefore, it is a very promising material for development of injectable hydrogels.

The goal of this study was to develop injectable hydrogels containing solubilized dECM isolated from porcine bone (bdECM).

Porcine tibia was demineralized using hydrochloric acid (at room temperature (RT) and 4 °C) and decellularized by means of either Triton X-100 (at RT and 4 °C) or trypsin (at 37 °C). The concentration of DNA, GAGs and collagen was studied after hydrolysis of the freeze-dried bdECM powder. Additionally, content of BMP2 and VEGF was determined by ELISA after extraction with guanidine hydrochloride. The obtained bdECM powder was dissolved by pepsin digestion at concentration of 10, 20 and 30 mg/ml and rheological, gelling and mechanical properties of the solutions and the corresponding hydrogels were studied using rheometer. The biological evaluation was performed with human mesenchymal stem cells (hMSC) and pepsin-digested type I collagen hydrogels used as a reference biomaterial.

The biochemical analysis revealed that the composition of bdECM varied depending on the demineralization and decellularization temperatures, with demineralization carried out at RT and decellularization at 4 °C yielding the lowest DNA content. Rheological tests showed concentration-dependent increase in dynamic viscosity and shear thinning behavior of all pre-gel solutions. The sol-gel transition took approximately 10 mins after incubation at 37 °C. Moreover, we observed inversion of the viscoelastic properties from viscous to elastic with increasing concentration of dECM. The ability of hydrogels to support hMSC proliferation and osteogenic differentiation was confirmed by means of MTS, DNA and ALP activity assays.

keywords: decellularization, injectable hydrogels, bone tissue engineering, MSC, rheological properties

20941814124

EXTRACELLULAR VESICLES CONTROLLED DELIVERY FROM GELLAN GUM-BASED HYDROGEL IN REGENERATIVE MEDICINE

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Introduction:
Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) play an important role in cell physiology, cell-cell communication, and immunomodulation. EVs are promising for regenerative medicine1, but their therapeutic effect in vivo is reduced due to their rapid clearance and short half-life2.
Injectable hydrogels emerged as great bioactive compound carriers due to their ability to mimic the extracellular matrix (ECM) and provide an accurate control on release3. These systems can be made of different polymers, including gellan gum (GG), a biocompatible bacteria-derived polysaccharide4.
In the present work, we develop a novel delivery system consisting of a gellan gum-based hydrogel to achieve a controlled release of EVs derived from human adipose mesenchymal stem cells (hA-MSC). We then evaluated the cross-talk between the EVs, hA-MSC, rat hippocampal neural stem cells (rH-NSC), and human skin fibroblasts (WS1).

Methodology:
Gellan gum-based hydrogels were fabricated by using bivalent ions, including magnesium and calcium ions. The resulting system was also implemented with hyaluronic acid (HA) (weight ratio between GG and HA from 1:4 to 1:1) and spermidine (ratio between polymers and spermidine equal to 1:5), a biocompatible crosslinking agent able to enhance the hydrogel stability and endowed with anti-inflammatory properties5. The resulting samples were characterized using rheology to evaluate the gelation time and mechanical properties, the stability and the swelling ability of hydrogels were investigated up to 21 days after the incubation in physiological-like conditions.
EVs were isolated from hA-MSC by an ultracentrifugation protocol6 and loaded within the hydrogels7. To evaluate hydrogel biocompatibility a preliminary screening on rH-NSC, WS1, and hA-MSC was performed analyzing cell viability and cell morphology up to 7 days. In addition, MSCs-derived EVs release from the hydrogel, and the resulting cross-talk effect was tested by real-time PCR.

Results:
The hydrogels showed a dependence of gelation time, stability, mechanical properties, and swelling degree on their composition. Hydrogels exhibited good biocompatibility without affecting cell viability and morphology of all the screened cell cultures. MSCs-derived EVs were homogenously embedded into the hydrogel without negatively affecting the gelation kinetic and they were released in a sustained and controlled way, additionally, they maintained their structure and bioactivity enhancing the cell viability.

Conclusions:
In the current work, we developed a fully bioresorbable and biocompatible controlled delivery system for EVs. This system is able to overcome issues that normally are faced in vivo. In fact, it might prolong the retention of EVs and maximize the localized benefit in situ. The resulting system displayed good biocompatibility on human fibroblasts, hA-MSC, and rH-NSC and is a promising system for several regenerative medicine applications.

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keywords: hydrogel, delivery system, extracellular vesicles, gellan gum, regenerative medicine

20941816305

MATERIAL-ASSISTED BIOENGINEERING STRATEGIES FOR OSTEOCHONDRAL DEFECT REPAIR

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The osteochondral (OC) unit, composed of cartilage and subchondral bone, plays a pivotal role in joint lubrication and in the transmission of constraints to bones during movements. The OC unit can be damaged by repetitive excessive loading, trauma or diseases, leading to cartilage defects. As OC defects does not spontaneously heal, different surgical approaches have been developed (e.g. chondroplasty, arthroscopic lavages and debridement, autologous chondrocyte implantation (ACI), matrix-associated ACI) and several biomaterials have now reached the market. Although present curative care temporarily improves joint functions, it often leads to the formation of fibrocartilaginous repair tissue, the deterioration of the subchondral bone, and functional loss in the long term. OC defects are therefore considered to be one of the major risk factors for long-term degenerative joint disease development such as osteoarthritis. In this context, a plethora of tissue engineering strategies have been envisioned, combining cells, biological molecules and/or biomaterials. A comprehensive study of biomaterial-assisted bioengineering strategies that have been tested in OC defect between 2015 and 2021 was performed. The analysis of almost 300 studies provided a deeper insight into the field, with careful considerations for the therapeutic potential of the different strategies, and current OC regeneration evaluation methods. This study highlighted the multiplicity of strategies that have been envisioned, along with the unlimited number of possible combinations of biomaterials, cell types and bioactive molecules.

The careful analysis of the current state of the art has revealed the emergence of promising strategies, including injectable multilayer and gradient materials, which have shown remarkable improvement in neocartilage and subchondral bone quality, supporting the use of more biomimetic architectures. In parallel with advanced biomaterial design, the controlled release of bioactive molecules, including GFs, almost systematically improved outcomes compared to biomaterials alone. These strategies stimulate cell invasion and allow a shift to cell-free strategies, which is another promising approach to more easily meet the stringent quality and regulatory requirements for safety and efficacy needed for clinical application. Particular attention should be paid to the use of common and more comprehensive assessment methods (scoring systems, assessment methods and animal models), following clear guidelines agreed by the international community. Therefore, though our comprehensive study of biomaterial-

assisted bioengineering strategies, this present work will help to build a better common knowledge and be a first step toward progressing in OC regeneration.

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keywords: osteochondral defect, cartilage, subchondral bone, regenerative medicine, biomaterials, cell therapy, biologics

94238119324

POLYLACTIC ACID-POLYCAPROLACTONE COPOLYMER NANOFIBERS FOR ANTIFIBROTIC DRUG DELIVERY

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Although fibrosis is a natural process with a protective function in the body, it can lead to progressive disease and eventual loss of organ function. This pathology can affect different tissues, ranging from skin to the heart; indeed, lung fibrosis has been observed in COVID-19 patients. The proposed root causes of fibrosis are injury, infection and chronic inflammation, which leads to hyperactivation of myofibroblasts. These produce an abundance of collagen to the extracellular matrix (ECM) and are also considered to maintain the inflammatory state in the tissue. Treating fibrosis is complicated because the affected tissues are poorly permeable to pharmaceuticals. Current treatment involves oral intake of medication, which often leads to side effects. We propose an advanced way of delivering an antifibrotic drug directly to the site of affected tissue using a polylactic acid (PLLA)-polycaprolactone (PCL) copolymer. Using this carrier would ensure prolonged and focused release of the pharmaceutical, potentially improving treatment and reducing negative side effects.

WPMY-1 prostate cancer myofibroblasts (ATCC) and primary human myofibroblasts (HMF) were used in this study. The latter was isolated from urethral scar tissue and confirmed to have the α-smooth muscle actin myofibroblast marker via immunocytochemistry. The drug release profile of the copolymers (pure PCL, 10:90, 35:65, 15:85 PCL:PLLA composition) was evaluated over a 4 month period. The antifibrotic drug-laden PLLA-PCL fiber cloth was manufactured by electrospinning; the products were investigated by evaluating their manufacturing capabilities. The effect of the copolymer carrier was analyzed in vitro in two ways. First, the viability of the carrier-drug affected myofibroblast cells was evaluated by the MTT test. Second, gene expression levels of fibrosis-related genes (Tgfb1, Timp1, Pai1, Ctgf, Vegfa and Mmp1) was determined, comparing pre-treatment and post-treatment levels.

The 10:90 PCL:PLLA polymer showed optimal release (concentration and time-wise) of the antifibrotic drug. Our data showed that using this carrier would maintain an effective concentration of the drug in the medium that would be within the therapeutic range, as revealed by the gene expression results. The effect was more pronounced in the WPMY-1 cell line than primary human myofibroblasts, but both showed satisfactory in vitro results.

We conclude that using the drug-laden PCL:PLLA copolymer could be a prospective avenue for treatment. In vivo testing could be the next step for bringing this technology into practice.

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keywords: Myofibroblasts, fibrosis, copolymer, drug delivery

94238103455

SELF-HEALING HYDROGEL WITH MICELLAR ARCHITECTURE FOR NEURAL REPAIR

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Injured neural tissue after the intracerebral hemorrhage (ICH) stroke has limited endogenous regeneration because of inflammation and cell apoptosis. The brain cavity and neural scar tissue caused by the hematoma often lead to a permanent behavior disability. Biomaterials can serve as temporary supporting matrices and promote brain tissue regeneration. A chitosan-based self-healing hydrogel (CM hydrogel) with micellar structure and comparable stiffness (modulus ~ 0.5 kPa) to brain tissue was developed based on modified chitosan and an aldehyde-terminated triblock copolymer crosslinker. The hydrogel network with self-healing ability was built up through Schiff-base reaction and filled with self-assembled micellar cavities (~20 nm) constructed by the hydrophobic interaction. Two model drugs with opposite affinity to water were successfully packaged (100% efficiency) in the hydrophilic network and hydrophobic cavities of the hydrogel, respectively. Such a drug delivery hydrogel showed two different releasing kinetics. The kinetics included a first-order rapid releasing tendency to the hydrophilic drug and a zero-order sustained releasing to the hydrophobic one. In the stroke model, a hydrophilic anti-inflammatory drug for short-term release and a hydrophobic neuroprotective drug for long-term release were selected in accordance with the subacute and chronic phases of stroke pathology. After 2 weeks of the hydrogel injection into the stroke rats, the scar tissue nearby the stroke cavity significantly decreased, while the signal of doublecortin, a marker from new nerve cells, was observed through immunohistochemistry staining. Magnetic resonance imaging (MRI) revealed the reduced inflammation around the brain cavity in the experimental group. The behavior performance of rats was improved after 28 d of the therapeutic period, evaluated by the rotarod test. The new self-healing hydrogel with micellar architecture offers as a novel drug delivery platform for diseases with complex time-dependent pathological stages, such as the ICH stroke.

keywords: self-healing hydrogel, drug delivery, intracerebral hemorrhage (ICH)

94238139048

THE BIOPACER- A BIOLOGICAL SOLUTION FOR THE RESTORATION OF HEART RATE IN PEDIATRIC PATIENTS

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Introduction
The atrioventricular block constitutes a common congenital disease among pediatric patients. Nowadays the treatment requires repetitive operations for the placement and replacement of technical pacemakers influencing the life quality of young children. In our project, we developed a tissue engineered solution with the aim to avoid the technical pacemakers and therefore the repetitive operations.

Methods
The preparation of the constructs included the fibrin gel embedding of 3 cell types, smooth muscle cells, endothelial and iPSCs-cardiomyocytes constituting the Biopacer1. In particular, the moulding of the Biopacer into a homogeneous tubular matrix made of lyophilised fibrin and silk fibroin was also studied. The lyophilised shield aims to protect the construct from degradation during the future implantation process. The Biopacer was cultivated for various time periods and a diameter of either 0.5 mm or 1 mm to assess the potential functionality of the construct. Cyclic stress was applied in order to increase the extracellular matrix protein formation induced mainly by fibroblasts to strengthen the inner core of the construct. For the evaluation of the constructs, there were glucose and lactate measurements performed. The constructs were also microscopically examined for the determination of the beating frequency of the iPSC-cardiomyocytes. Moreover, the Biopacer was tested for the expression of markers owning to the 3 cell type components.

Results
The glucose and lactate analysis showed that the 0.5 mm diameter Biopacer had a greater lactate production than the 1 mm construct at the end of the cultivation period, while the end glucose levels remained the same. The expression of smooth muscle actin (αSMA) indicating the effect of smooth muscle cells and the CD-31 the effect of endothelial cells respectively, showed that the remodelling in the 1 mm Biopacer construct is functioning. At the same time, the expression of the sarcomeric α-actinin (SAA) indicating the maturity of the iPSCs-cardiomyocytes reflected the maturity of the cells in both diameter constructs and was higher in the 1 mm Biopacer as attributed to the iPSCs-CM number. The beating frequency of the iPSCs-CMs was calculated higher in the fibers containing only iPSCs-CMs while the respective ones of the 0.5 mm and 1 mm were wide declined.

Conclusions
The cell composition of the constructs and the diameter strongly affect the functionalisation of the Biopacer in terms of the lactate production levels and beating frequency. The expression of the iPSCs-CM markers gives us sight to the implementation of the method principles to proceed

with the optimisation of the protective shield.

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keywords: Atrioventricular block, iPSC-derived cardiomyocytes, Fibrin hydrogel

PS40
Injectable scaffolds in tissue engineering

20941856328

CELL ENCAPSULATION IN PHOTOCROSSLINKED ALGINATES: MECHANICAL CHARACTERIZATION AND CELL VIABILITY STUDY

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In the past few years, mesenchymal stromal cells (MSC) have been identified as a potential therapy to inflammatory diseases due to their ability to secrete immuno-modulatory and anti-inflammatory factors. Considering the risk of cell leakage outside the target site and the poor survival rate after a local injection, for example in the joint, cell encapsulation in hydrogels could overcome these limitations, and provide cells a suitable 3D microenvironment supporting their biological activities. Sodium alginate is the most frequent natural polymer used for cell encapsulation, and is crosslinked by addition of divalent cations such as Ca2+ (AlgCa). As such, a physiological environment with monovalent cations, such as Na+, might impair the stability of ionically crosslinked alginate. In this context, we hypothesized that MSC could be encapsulated in a more stable photocrosslinked methacrylated alginate (AlgMA) hydrogel.

Four degrees of methacrylation of AlgMA were tested (targeting degrees of substitution of 10, 25, 50 and 75%). Photopolymerisation of AlgMA was performed with a photo-initiator (Irgacure® 2959) under UV-A light exposition (365 nm), with concentrations of AlgMA and Irgacure of 2% and 0.05% (w/v), respectively. AlgMA solutions were exposed to UV-A light intensities of 8.9 mW/cm^2 (low) or 21.7 mW/cm^2 (high), and were compared to AlgCa hydrogels crosslinked with 100 mM CaCl2 and stored in 100 or 1.8 mM CaCl2 for 24 hours. The effect of the methacrylation degree on the stiffness of the photocrosslinked AlgMA gels was investigated, via compression tests (Microtester, CellScale). Hydrogel stability in Dulbecco's Modified Eagled Medium (DMEM), ultrapure water and sodium citrate 80 mM, was assessed for AlgMA and AlgCa hydrogels with weight analysis during 15 days. Human adipose-derived MSC viability in AlgMA hydrogels was analyzed by LIVE/DEAD® assay for up to 15 days. All assays were performed on cylindrical hydrogel punches of 2 mm diameter and 1 mm height.

AlgMA hydrogels stiffness significantly increased with methacrylate substitution and duration of exposure to UV-A light, with no significant difference observed over 40 minutes of high intensity exposure, reaching 190 kPa. By comparison, the elastic moduli of AlgCa hydrogels were similar to the one of 50% and 75% AlgMA crosslinked at high UV-A intensity for only 6 and 2 minutes respectively (< 15 kPa). In ultrapure water the weight and the elastic modulus of the AlgMA

hydrogels increased significantly, whereas they remained stable in culture medium for 15 days. AlgMA hydrogels did not dissolve in sodium citrate, unlike AlgCa hydrogels. The cell viability study showed that in all the AlgMA tested conditions, excepted 10% AlgMA, the encapsulated MSCs were viable (viability higher than 70%).

Human MSC have been encapsulated in alginates with four degrees of methacrylation. We confirmed that AlgMA hydrogels are more stable than AlgCa ones in a physiological environment containing sodium ions. All, excepted 10% AlgMA were suitable for cell encapsulation. Further in vitro experiments are under investigation to characterize the diffusion of immune-modulatory and anti-inflammatory factors and to determine whether encapsulated MSC in AlgMA hydrogels may be a relevant strategy to treat inflammatory diseases.

keywords: Cell therapy, hydrogel, Young modulus

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ELASTIN-LIKE POLYPEPTIDE-BASED BIOINK FOR HUMAN SKIN DERMAL COMPARTMENT RECONSTRUCTION

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Three-dimensional (3D) bioprinting offers a great alternative to traditional manual techniques for tissue reconstruction in terms of time consumption and reproducibility. When suitable bioink is engineered with appropriate physico-chemical properties, such process can advantageously provide a spatial control of the patterning that improve tissue reconstruction. The design of an adequate bioink must fulfill a long list of criteria including biocompatibility, printability and stability. With the purpose to reconstruct a human skin dermal compartment, we have developed a bioink containing a precisely controlled recombinant biopolymer, namely elastin-like polypeptide (ELP). These materials provide a 3D network through photopolymerization. ELP chains were additionally either functionalized with a peptide sequence GRGDS (Gly-Arg-Gly-Asp-Ser) or combined with Collagen I to enable cell-adhesion. Our ELP-based bioinks were found to be printable in a short period of time, while providing excellent mechanical properties such as stiffness and elasticity in their cross-linked form. Besides, they were demonstrated to be biocompatible showing viability and adhesion of dermal normal human fibroblasts (NHF). Expressions of specific ECM protein dermal markers as Pro Collagen I, Elastin, Fibrillin and Fibronectin were revealed within our human dermal reconstructions, showing the great potential of ELP-based bioinks for skin tissue engineering.

keywords: Recombinant elastin-like polypeptide, 3D bioprinting, Bioink, Tissue engineering

20941835766

HISTOLOGICAL REVIEW OF MICROSPHERE SCAFFOLD IMPLANTS FOR ARTICULAR CARTILAGE REGENERATION IN A PORCINE MODEL

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INTRODUCTION
Articular cartilage has excellent biomechanical properties, but due its avascular nature and the low proliferation capacity of mature chondrocytes, the regeneration process usually produces fibrocartilage scars, with worse biomechanical properties. For this reason, articular cartilage traumatic injuries and degenerative pathology are frequent causes of pain and disability. Currently, tissue engineering techniques are expanding for the treatment of these injuries, through the design of scaffolds that support the chondrocytes while ensuring enough resistance to allow the limb to bear weight but not isolating them, since tensions are important signals for differentiation and formation of hyaline extracellular matrix.
Our objective was to design and synthetize a three-dimensional scaffold in the form of microspheres and implant it in a porcine animal model to evaluate the quality of the newly formed tissue.

METHODOLOGY
We used microspheres synthetized from two different biomaterials: polylactic acid (PLA) and platelet-rich plasma (PRP), since this type of scaffold showed good results in a rabbit model. To keep the microsphere scaffold in place, we also used a 100 µm PLA membrane.
Surgical interventions were performed in a porcine experimentation model, creating a full-thickness chondral lesion, 6 mm in diameter, in the weight-bearing part of the medial condyles (n = 24 knees). Three different treatment groups were formed: (a) control group without microspheres, covered with PLA membrane, (b) control group with a Novocart (Braun®), an approved treatment for cartilage lesions in humans, and (c) experimental group with PRP and PLA microspheres, covered with PLA membrane. In addition, microfractures were performed on the subchondral bone in all groups, before assigning them to a treatment group.
Ten months after surgery, the femurs were obtained and processed with the histological technique. Haematoxylin-eosin staining was used, and the histological analysis was performed with the ICRS II scale, which was modified by adding three items: microsphere presence, membrane presence and inflammation around the area of the remaining biomaterial.

RESULTS
In most ICRS II scale items, the experimental group with microspheres and the Novocart control group had similar results, with a tendency to have better results than the control group without microspheres. We found no traces of biomaterial in any sample of the microspheres group, while they were found in 2 of the 8 Novocart samples. The global result of the ICRS II scale had a similar trend in the experimental and Novocart groups. There were no inflammatory areas around biomaterials used.

CONCLUSION
The overall ICRS II score appears to be similar when comparing the PRP and PLA microsphere scaffolds group with the Novocart group, an already approved treatment for articular cartilage injuries. No microspheres were found in any sample after 10 months, which could show that they are reabsorbed at an adequate rate to add support for chondrocyte migration without leaving debris in the newly formed articular cartilage.

keywords: Scaffolds, Microspheres, Articular Cartilage, Musculoskeletal, Porcine model.

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IN-SITU GELLING HYDROGELS BASED ON OXIDIZED POLYSACCHARIDES AND GELATIN FOR TISSUE REGENERATION

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In previous decades, biomedical research has focused on the development of biomimetic and biocompatible materials, that do not cause any adverse reactions in the human body [1]. Synthetic materials are already widely used, but usually lack the necessary bioactive cues to promote a specific cellular response. Also, the degradation of these materials can cause toxic side effects. Polysaccharidesare biocompatible and bioactive natural polymers, which can be used as building blocks of scaffolds and hydrogels in the field of tissue engineering and regenerative medicine. Here we propose the development of a hydrogel based on oxidized polysaccharides containing aldehyde groups (like hyaluronic acid and alginic acid) and modified gelatin (HYD-Gel)carrying hydrazide groups. The amine and hydrazide groups react with the aldehyde groups forming irreversible hydrazine bonds. As a result in-situ gelling, cell laden hydrogels can be synthesized and used as a scaffold without the need of a potentially toxic crosslinker. Gelatin is specifically chosen to improve the bioactivity of the scaffold, while the native polysaccharides and their degradation products show a high biocompatibility. Under physiological conditions, these hydrogels are supposed to degrade slowly, while the cells proliferate and slowly replace the scaffold. Preliminary studies have shown that the variations in the component concentration and ratio lead to hydrogels with different properties and degradation times. This adjustability can become important, as different cell types have divergent requirements for the scaffolds in terms of elasticity and degradability. In vitro studies with similar hydrogel systems based on oxidized polysaccharides have already demonstrated their cytocompatibility in the presence of human adipose derived stem cells [2]. As the precursors of the hydrogels will gelate almost instantaneously when coming in contact with each other, they additionally can be used as bioinks in the formation of 3-D scaffolds.

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keywords: polysaccharides, hydrogel, scaffold, tissue engineering

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PHOTOCROSSLINKED HYALURONIC ACID-BASED HYDROGEL COMBINED WITH PRP FOR ARTHROSCOPIC CARTILAGE REPAIR

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Introduction
Standard surgical procedures such as debridement and microfracture in combination with application of various biomaterials into defect were suggested to support cartilage healing. Although these techniques provide short-term relieve from pain and temporary restoration of joint function, long-term studies discovered that healing process led to formation of fibrocartilage with high content of COL1 instead of COL2. Therefore, the most important improvement that TERM approaches should bring to cartilage treatment is to support formation of hyaline cartilage with high content of COL2.
Hyaluronic acid is a natural polymer which is often used for treatment of cartilage defects. Tyraminated derivative of hyaluronic acid (HA-TA) was proved to be a suitable material for preparation of in situ gellable hydrogels which enable cell encapsulation [1,2]. HA-TA-based scaffolds support chondrogenic differentiation of surrounding cells but don´t support cell attachment. On that account, combination of these scaffolds with patient´s own platelet-rich plasma (PRP) is gaining attention. Plasma proteins serve as a source of adhesion molecules; cytokines and growth factors participate in directing cell differentiation.
Here we present a fully biocompatible photocrosslinkable HA-TA-based hydrogel which allows combination with PRP plasma and can be applied during arthroscopic surgery and solidify in situ after exposure to arthroscope light. Initiation of photocrosslinking reaction by visible light is enabled by photosensitizer riboflavin.

Methodology
Influence of HA-TA concentration (2 % or 3 % (w/v)) and PRP concentration (12,5 %, 25 % or 50 % (v/v)) in hydrogel on cell growth and differentiation was tested in vitro.
HA-TA was dissolved in riboflavin solution overnight, sterilized in autoclave and mixed with human PRP. This mixture was injected in culture wells and solidified by irradiation by arthroscope light for 3 min. Chondrocytes were seeded on top of hydrogel layers and cultured in standard culture medium for 14 days. Cell proliferation and gene expression were assessed. Hydrogels were characterized by determination of viscoelastic properties and swelling ratio.

Results
Both 2 % and 3 % HA-TA hydrogels containing PRP supported chondrocyte attachment and increased COL2/COL1 ratio compared to control, but hydrogel with higher HA-TA content was less stable due to higher swelling.
Concentration of PRP in 2 % HA-TA hydrogels influenced cell growth and gene expression: cell attachment, proliferation and production of COL1 were highest on hydrogels containing 50 % PRP and lowest on hydrogels with 12,5 % PRP. Production of chondrogenic differentiation-related genes COL2, SOX9 and ACAN was highest on hydrogels containing 25 % PRP.
Therefore, 2 % HA-TA and 25 % PRP appears to be the optimal composition of hydrogel which supports both cell growth and chondrogenic phenotype.

Conclusion
We developed a photocrosslinkable HA-TA-based material for treatment of cartilage defects which can be applied during arthroscopic surgery. Using riboflavin as a photosensitizer allows in situ solidification of the material after irradiation by arthroscope light. Developed material allows combination with PRP plasma and supports cell adhesion and chondrogenic differentiation.

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keywords: hyaluronic acid, photocrosslinking, hydrogel, arthroscopy, cartilage

73296325688

SMALL COMPOUND RELEASE FROM INJECTABLE NANOFIBROUS MICROSCAFFOLDS

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Recent regenerative medicine is highly focused on therapies that can effectively treat intravertebral disc (IVD) degeneration. Because of its high efficiency, tissue engineering is a promising alternative therapy for cartilage defects and IVD is one of its main targets. Here we report on the fabrication of injectable electrospun microscaffolds (MS) containing a small chondrogenic agent: TD-198946.
Drug-loaded nano-/microfibers were prepared using the electrospinning technique. The therapeutic agent was incorporated directly, by solubilizing into a polymer solution to be spun. Then, fibers were structured with a picosecond laser to form thousands of 100 um in size microscaffolds. Morphology of MS was analyzed by Scanning Electron Microscopy (SEM) in order to characterize the impact of laser processing on the polymer fibers and cell morphology in contact with MS.
The main problem with drug delivery from polymeric vehicles is the initial burst release which is often unavoidable and can be controlled by many parameters e.g. temperature or pH. We performed drug release studies from both unstructured fibers mat and MS in different pH 6.4 and 7.4, which is characteristic for degenerated and normal/healthy tissue. Studies were carried out for 120 and 60 days and around 80% and 100% of the drug was released from fibers mat and MS, respectively. The differences in the release rate were also observed between various pH.
We developed a novel and straightforward method to fabricate microscaffolds from almost any type of electrospun material. PLGA-based MS containing drug molecule TD-198946 showed a controlled and gradual release profile which was slightly faster compared with unstructured material. The developed vehicle is supposed to be applied in IVD degeneration, where drug-loaded MS enhances the synthesis of glycosaminoglycans. A single administration of the material to the tissue will result in several weeks of substance release, which may have a beneficial effect on the regenerative processes of the IVD.
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keywords: drug release, nanofibers, electrospinning, IVD

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TUNABLE HYBRID 3D PRINTING CRYOGELS - A PERSONALIZED VEHICLE TOWARDS BONE REGENERATION

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Despite key limitations such as host bone supply and quality, donor site morbidity, and immunogenicity, autologous and allograft bone are still considered the clinical standard of care for bone repair [1]. In addition, biomaterials for bone regeneration should be not only biocompatible and osteoconductive, but also osteoinductive [1, 2]. Developing a biomaterial that comprises all the key features all-in-one, such as: i) improve vascularization, ii) bioactivity towards tissue regeneration, iii) easy appliance and iv) avoid host rejection, remain a major research topic nowadays. The development of such material often lacks in particular aspects, like reduced nutrient diffusion or cell perfusion, lack of biological cues, no organized structure or resemble the target tissue and last the difficulty to develop the biomaterials which drawbacks the translational into the clinic. Here we propose the development of a 3D-printed nanocomposite cryogel, based on laminarin combined with bioactive mesoporous silica nanoparticles, with an anisotropic topology achieved by a simple freeze casting method. This lamellar topography resembles the bone structure and it's supposed to favor vascularization [3]. The cryogel characteristic porosity structure allows a better nutrient diffusion, cell perfusion and offers the possibility to be injected. In addition, the cryogels excel in their mechanical features, once they present high stiffness and toughness, and no obvious recovery loss at 40% strain, these characteristics match those of targeted implantation tissue, which are stiff but not fragile. It is also demonstrated that the presented cryogel method is more advantageous than the commonly used technique of freeze-drying a hydrogel. Overall, the macroporous cryogels show a better recovery, with no deformation of pores, while the freeze-dried cryogels, after suffering load and unload compression cycles, exhibit a tendency to squeeze and lose their porosity. Lastly, the nanoparticles enclosed on the cryogel matrix allow us to enhance the bioactivity, suitable for bone regeneration. Resorting an SBF assay, the loading of bioactive silica nanoparticles into the cryogels enhanced greatly hydroxyapatite growing on top of the scaffolds. Our results demonstrated that these materials are highly reproducible, easy castable into multiples structures, injectable, bioactive, encompassing an oriented structure, and as far as the authors know, are the first 3D-printable anisotropic nanocomposite cryogels to be reported. We anticipate that this study prompts the development of a different array of anisotropic nanocomposite cryogels, covering more complex structures, helping the scientific community reach a step further to a clinical application respecting bone regeneration.

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keywords: Cryogels, bone regeneration, anisotropic pores, 3D printing, bioactive scaffolds

PS41
Mesenchymal Stem / Stromal Cells - from basic research through clinical studies to registered products

73296312817

3D IN VIVO BONE MARROW ORGANOIDS TO DISSECT MESENCHYMAL STROMAL CELLS CHAOS

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Introduction
The bone marrow (BM) is a dynamic organ whose main function is to support haematopoietic cells, which assure life-long production of blood. The haematopoietic compartment is supported by the stromal fraction of the BM that comprises mesenchymal stromal cells (MSCs), a heterogeneous cell population that undergoes osteogenic, chondrogenic, adipogenic differentiation. Following a tissue engineering approach, we combine the knowledge on BM-MSCs with biomaterials to dissect such cells heterogeneity and model BM biology. Our technology consists in encapsulating BM-MSCs in synthetic, biocompatible polyethylene glycole (PEG) hydrogels and following cells developmental potential in vitro and in vivo. We are currently focusing on the in vivo characterization of two murine BM-MSCs populations, Skeletal Stem Cells (SSCs) and Bone Cartilage Stromal Progenitors (BCSPs), aiming at generating new knowledge for bone and bone marrow regenerative strategies.

Methods
SSCs and BCSPs were mechanically and enzymatically extracted from limbs and sternum of post-natal day3 (P3) GFP+-C57/BL6 mice and separated by FACS using the marker panels CD45-Ter119-Tie2-AlphaV+Thy-6C3-CD105-CD200+ and CD45-Ter119-Tie2-AlphaV+Thy-6C3-CD105+, respectively. Due to the high amount of cells needed for in vivo trials, both cell types were expanded under standard culture conditions for 7 days. Subsequently, the cells were encapsulated at different cell densities in PEG hydrogels with or without minimal amounts of BMP-2. Such scaffolds, whose size is around 0.5 cm in diameter, were subcutaneously implanted at the back of NMRI-Foxn1nu immunocompromised mice and harvested after 8 weeks. Upon explantation, samples were macroscopically inspected, imaged and fixed. Bone formation was quantified through micro-CT analysis, followed by decalcification and histological characterization.

Results
The subcutaneous implantation of the scaffolds allowed to visually monitor their localization within the 8 weeks of implantation. At explantation, all the scaffolds were found back with their initial size, confirming biomaterial stability. Distinct scenarios in terms of BM organoids (or ossicles) were observed based on the different combinations of cell densities and BMP-2. A first macroscopic evaluation showed SSCs being more performant as they induced the formation of stiffer ossicles with high degree of vascularization both with and without BMP-2, at different cell densities. BCSPs generated ossicles only when in combination with BMP-2. Still-ongoing Micro-CT and histological analysis will precisely determine whether there is a direct proportionality between cell density, BMP-2 concentration at implantation and bone formation or marrow

morphological complexity. Also, the GFP-labelling of the implanted cells will aid tracing them back in the ossicles, giving further information on their role in the BM organoids onset, thus on their biological function.

Conclusions
The results suggest that our technology can generate 3D in vivo BM organoids and so elegantly model such organ. Moreover, it proved to be a good instrument for investigating SSCs and BCSPs biological function in vivo, since even from a first macroscopical evaluation different outcomes from SSCs or BCSPs-loaded scaffolds were observed. Lastly, the biocompatibility of our biomaterial was demonstrated as most of the ossicles were found to be vascularized, thus suggesting their integration with the host vasculature and so the remodeling of the material by implanted or host cells.

keywords: Biomaterials, In vivo organoids , Bone , Bone Marrow , MSCs

41883618808

CELL THERAPY FOR OSTHEOARTRITIS: EFFECTS AND MECHANISMS OF ACTION

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Introduction
The overall burden of osteoarthritis (OA) in the US is estimated at 6.8B annually, an amount that surpasses that of tobacco-related health effects, cancer, and diabetes. Current interventions are not effective in arresting the progressive cartilage degeneration. Stem cell therapy is the new frontier for OA treatment. Intra-articular injection of mesenchymal stem cells (MSCs) derived from different tissue sources has demonstrated its potential to support regeneration of damaged cartilage, thus preventing OA progression. Adipose-derived Stromal Cells (ADSC) are one of the potential candidates for cell therapy. The molecular mechanisms of ADSC-cartilage interactions remain uncertain. We used an ex vivo model of cartilage degeneration to assess metabolism of ADSCs and their effect on diseased cartilage.

Methodology
ADSC were harvested with the use of disposable closed loop device, Mini-Stem System (Jointechlabs), from donated human lipoaspirates following patients consent. Harvested cell fraction was analyzed by FACS and cell proliferation was assessed by Alamar Blue assay and in culture. Full thickness 4mm cartilage explants from normal ankle joints of 11 human organ donors (9 males and 2 females; age 45-75 yo) were pre-cultured in the presence of IL-1β (1ng/ml or 10ng/ml) to mimic pro-inflammatory environment of the joint. The explants were the incubated with ADSCs encapsulated or not within proprietary hydrogel. The regenerative secretome released by ADSCs was assessed by multiplex ELISA for a representative panel of catabolic and pro-anabolic cytokines. The effect of the secretome on cartilage was evaluated by cell viability, necrosis, and gene expression for cartilage-specific genes. 2 million cells/well were used for culture with cartilage explants. Proteoglycan (PG) content and synthesis in ADSC-cartilage cultures was measured on days 0 (baseline), 3, 10, and 24 and normalized to DNA content using Hoechst 33258 dye. Analysis of pro- and anti-inflammatory mediators released into the culture media was done by Luminex multiplex ELISA of 15 Human cytokines and growth factors.

Results
We found that gel encapsulation provided an adequate support for ADSCs expansion at both high (1 million) and low (250,000) concentrations and that ADSCs survived pro-inflammatory environment. The striking effect of ADSCs on chondrocyte metabolism was identified with PG synthesis. ADSCs alone or embedded in hydrogel induced/elevated PG synthesis above culture control levels ($p=0.033$ and $p=0,036$ respectively) and restored PG synthesis inhibited by IL-1β ($p=0.058$ and $p=0.004$ respectively). ADSCs also elevated an IL-1RA/IL-1 ratio by 4-fold alone or 7-fold in hydrogels in comparison to IL-1β control ($p<0.001$).

Conclusions
This study demonstrated that ADSC exhibited 1) a pro-anabolic activity by restoring and stimulating above control levels PG synthesis inhibited by IL-1β and 2) a continuous anti-inflammatory response by upregulating IL-1RA and its ratio to IL-1β. This is the first time when ADSCs were shown to engage in a complex pro and anti-inflammatory paracrine interaction

with human adult chondrocytes in vitro. The results of this study suggest that ADSC survive an inflammatory environment, generate anti-inflammatory effect and restore synthesis of cartilage extracellular matrix components. This study supports the further development of the ADSC PoC cell therapy for pre-clinical and clinical trials.

keywords: Adipose-derived Stromal Cells, cartilage regeneration, osteoarthritis, inflammation, anabolic response

62825435046

CHARACTERISTICS OF MESENCHYMAL STEM CELLS ACTION ON EXPERIMENTAL INFLUENZA VIRUS-INDUCED PNEUMONIA

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Introduction
The studies of MSCs therapeutic action have been conducted over the past 20 years for a variety of human pathologies, both in model experiments in animals and in clinical trials. However, there are very few studies on the effects of MSCs in viral infections. Thus the present work aimed to study the effects of MSCs administration at the very beginning of the acute phase on the course of influenza virus-induced pneumonia in mice.

Methodology
Mice were inoculated intranasally by mouse-adapted influenza virus strain A/FM/1/47/H1N1. The virus infectious dose was 4,0 1g LD50. The next day, mice from experimental groups were injected with human umbilical cord MSCs via intravenous, intraperitoneal, or intranasal routes. The doses of MCS were 1x106, 2x105, and 2x104 cells per animal. The results were evaluated by: the survival rate of experimental animals compared with controls, the viremia, the state of internal organs, and their histopathological analysis.

Results
In the virus control group, all infected mice died 6 to 11 days p.i. While in the experimental group with intravenous administration of 1x106 MSCs 20% of the animals remained alive. In the experimental group administered with 2x105 MSCs survival increased to 40%. However, dose reduction to 2x104 MSCs led to an earlier onset of the death, and the survival rate dropped to zero. The survival rate in experimental groups with intraperitoneal injection of 1x106 or 2x105 of MSCs was 20%. On the contrary, the experimental group that received 2x104 MSCs via the intraperitoneal route exhibited the same survival rate as the group with the 2x104 MSCs administered parenterally. All mice in the group were dead in the same time frame. In the group administered 1x106 MSCs, the death of animals began already on the third day and ended in complete death of animals. However, doses of 2x105 and 2x104 MSCs provided 40% survival. Histopathological studies revealed that the lungs in dead animals from the experimental groups administered with MSCs were elastically compacted. Internal organs had macroscopic signs of acute venous plethora in the vessels of the systemic circulation. A morphological study of lung tissues in dead animals with all types of administration and doses of MSCs showed a general picture of lesions: hyperplasia of broncho-associated lymphoid tissue; infiltration by lymphoid cells of the peribronchial tissue; the presence of lymphoid cells in the alveoli; and abundance of lymphoid cells in the lumen of the bronchi up to the obturation of these lumens. There was proliferation and infiltration of lymphoid cells in the respiratory sections of the lungs; dystrophic - destructive changes in the cells of the tissues of the organ; filling of the lungs and bronchi with

serous-vascular exudate.

Conclusion
Thus, the results demonstrated that MSCs could increase survival in the lethal influenza virus-induced pneumonia model. The doses of MSCs and their administration routes were of decisive importance. The effectiveness of intranasal administration of MSCs was demonstrated. The administration of MSCs at an early stage of the acute phase of influenza virus-induced pneumonia changes the course of the disease.

keywords: MSC, routes of administration, doses, virus-induced pneumonia, influenza

62825424489

COMPARISON OF CEREBROPROTECTIVE ACTION OF MESENCHYMAL STROMAL CELLS OF DIFFERENT ORIGIN AND LYSATE FROM HUMAN WHARTON JELLY MSC IN POST-PERFUSION LESIONS OF THE SENSORIMOTOR CORTEX OF RATS

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INTRODUCTION
One of the main causes of stroke in acute cerebrovascular accident is ischemia, which begins with the formation of acute neuronal energy deficiency with subsequent activation of “ischemic cascade” reactions, which leads to irreversible damage in nerve tissue. But the restoration of perfusion of ischemic tissue contributes to the deepening of metabolic disorders in the brain, which leads to the formation of reperfusion damage. In view of this, it was expedient to characterize and compare the effect of different origin mesenchymal stromal cells (MSC) and lysate from human Warton jelly (WJ) MSC on neuroapoptotic changes in the sensorimotor cortex of rats in the conditions of model ischemia-reperfusion (IR) by flow cytometry – one of the best methods for assessing the fragmentation of neuronal DNA.

METHODS
An experiment was performed using Wistar 4-month-old rats (males) weighting 160-190 g, which underwent a transient bilateral 20-minute IR of the internal carotid arteries. After modeling the pathology, the animals were injected into the femoral vein (IV) with MSCs obtained from human umbilical cord WJ, human and rat adipose tissue. Other groups of experimental animals were injected intravenously with rat fetal fibroblasts in 0.2 ml of saline and lysate of WJ MSCs at a dose of 0.2 ml / animal. Control animals were injected intravenously with 0.2 ml of saline. The level of DNA fragmentation in the nuclei of neurons of the sensorimotor cortex of rats on the 7th day after ischemia-reperfusion was investigated by flow cytometry. The study was performed on a flow cytometer “Partec PAS”, Partec, Germany. An ultraviolet lamp was used to excite the fluorescence of the nuclear DNA label – diamidinophenylindole, 10,000 events were analyzed from each sample of nuclear suspension. Flow analysis of DNA fragmentation was performed using FloMax software (Partec, Germany). The statistical significance of the differences was assessed by Student’s t-criterion.

RESULTS
The study showed that in the groups with transplanted MSCs of different origin and MSC lysate, the intensity of DNA fragmentation in the nuclei of sensorimotor cortex neurons reliably decreased. In 7 days the decline was 1.8 - 2.6 times compared with group without treatment. Transplantation of MSCs derived from WJ and human adipose tissue, rat fibroblasts and MSCs from rat adipose tissue, as well as lysate from human WJ MSCs, had a similar cerebroprotective effect on rats with IR. This was indicated by a significant reduction of DNA fragmentation in the nuclei of the brain sensorimotor cortex neurons during study period by an average of 58.98%, 56.11%, 61.78%, 43.45% and 47.13%, respectively ($p < 0.05$).

CONCLUSIONS
Thus, the suppression of the intensity of neuroapoptosis in the sensorimotor cortex of rats under the action of various origins MSCs and lysate of human WJ MSCs, indicates the decrease of ischemic penumbra focus due to the preservation of the number of morphologically intact neurons and is one of the mechanisms of cerebroprotective action in postperfusion brain damage. The activity of cell lysate testifies in favor of the paracrine mechanism of therapeutic action of MSCs.

keywords: ischemia, reperfusion, MSC, DNA fragmentationn

52354576408

COMPARISON OF CULTURE REQUIREMENTS FOR CANINE AND HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS

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Treating immune-related diseases, which incidence is steadily growing, is still a challenge. Among the new treatments under development, mesenchymal stromal cell (MSC) transplants seem to be one of the most promising. Although MSC-based therapies are being implemented into the clinic, many aspects regarding these procedures requires further optimization. A new trend in translational medicine is to treat veterinary medicine as a bridge between research on diseases models in laboratory animals and human clinical trials. Dogs suffer from immune-related diseases which are similar to those in humans. One of them is anal furunculosis, which is considered to be a model of anal fistulas in Crohn's disease in humans. The objective of this study was to optimize the culture conditions of canine mesenchymal stromal cells isolated from umbilical cords (cUC-MSC) and to compare their requirements to human counterparts (hUC-MSC). Cells isolated from 4 canine UC and 4 human UC were used in this study. Human cells met standard identification requirements for human MSCs. Isolated canine cells expressed MSC markers like CD90, CD44 and did not express hematopoietic markers like CD45 and CD11b (flow cytometry). Additionally they expressed vimentin (immunocytochemistry). In standard growth medium (GM, composed of DMEM+10% foetal bovine serum) and on standard uncoated culture surface canine cells underwent rapid senescence. After the 3rd passage a mean of 50.9% of cells displayed features of in vitro aging (β-galactosidase assay, β-Gal). In contrast, only 8.7% of human cells were senescent in the same conditions after 3rd passage, p=0.008 in comparison to canine cells. The metabolic activity (MTT assay) under these basic growth conditions was also significantly higher in human cells than in canine cells (p=0.001). The addition of basic fibroblast growth factor (bFGF, 5 ng/mL), coating culture dishes with porcine gelatin (GEL) and combination of both factors (bFGF+GEL) were tested to establish favorable growth conditions for canine cells. The highest growth rate was observed in bFGF+GEL conditions. The average population doubling time amounted 4.5, 1.7, 2.9, and 1.3 days for cells cultured in GM, bFGF, GEL and bFGF+GEL, respectively. The β-Gal assay performed in canine cells cultured in bFGF+GEL conditions showed that, on average, 6.2% of the cells after 3rd passage were senescent, and this level was 8.1 times lower than that of cells grown in GM (p=0.02). To assess the tolerogenic activity, cUC-MSCs and hUC-MSCs were co-cultured with allo-activated human peripheral blood mononuclear cells. Both cell types increased the proportion of T regulatory cells (FoxP3 expressing cells) in the population of CD3+CD4+ cells. Obtained results demonstrate that cells isolated from canine umbilical cords have different culture requirements than human UC-MSCs. However, when cultured in the presence of bFGF and on gelatin-coated dishes, a sufficient number of cells from one canine umbilical cord can be obtained for transplantation into at least one canine patient. These results open up the possibility of testing the effects of cell transplantation in dogs with naturally occurring immune-mediated diseases. Such veterinary clinical trials might also help to improve MSC-based techniques in humans.

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keywords: Umbilical cord, mesenchymal stromal cells, canine, human, culture conditions

31412720646

DECIPHERING COLLAGEN-VII ROLE IN BREAST CANCER ASSOCIATED MESENCHYMAL STEM CELLS.

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Breast cancer (BC) is the most common cancer and the fifth leading cause of death according to World Health Organization. Surgery is the main treatment for BC. After primary cancer-mass excision, in a high percentage of the cases, some breast cancer cells (BCC) with tumorigenic potential remain dormant/quiescent. In worst scenarios, dormant-BCC lose quiescence and re-activate their tumorigenic program, what extend treatments due to cancer recurrence.
The dormancy-to-activation process and subsequent tumor progression rely on the BCC-individual specific phenotype and is strictly modulated by a concrete extracellular niche. BCC are capable to modify their tumorous microenvironment according to the tumor dynamics.
The extracellular matrix (ECM) is mainly secreted by cancer associated fibroblasts (CAF) and is a major component of the tumor microenvironment. Breast fibroblasts arise from multipotent breast adipose tissue stem cells (ASC) and surrounding connective tissues. ASC as mesenchymal stem cells, are recruited to the tumorigenic areas where still, their function on tumor microenvironment is still barely understand. There is agreement on cancer cells induce ASC-to-CAF transformation, event that provide the supportive extracellular microenvironment for tumor growth and have been associated with worst cancer prognosis.
We hyphotisized that the maintenance of the ASC´s proper function into BC niche would impact negatively BC progression. Up today, no treatments against CAF have been postulated but, systemic drugs against collagen or TGFβ1. Hene, we aim to answer whether a local and specific ECM could blunt BCC and ASC communication to overriding the ASC-to-CAF differentiation and then block BCC´ dormant-to-activation transition.

Among different other ECM components, we found collagen VII (ColVII), a marker of certain cancer cells´ quiescence and understudied on breast cancer, overexpressed in dormant BCC. To study role of the extracellular ColVII we used a recombinant human Collagen-VII (rhColVII) and we assessed MCF-7 proliferation. We coated our plates with 0, 2, 5, 10 and 20ng/ml rhColVII. MCF-7 transgenic line carrying GFP flag were plated on prior coated with different rhColVII concentration and we followed the cultures for 10 days. GFP signal was significantly decrease in the cultures growing on 10 and 20 ng/ml rhColVII when were compared with MCF-7 growing in vehicle or lower doses. We confirmed our results by area occupied by GFP and Ki67 staining. Surprisingly, single cell transcriptome banks and Collagen VII breast cancer tissue staining, showed that ColVII is highly expressed by stromal cells when they were compared with cancer cells and these stromal stem cells loss ColVII expression along with markers of stromal stem cells as PDGFR-alpha when are exposed to BCC secretome.
Our preliminary results suggest that ColVII is expressed mainly by cells with stromal origin in the breast tissue. ColVII is overexpressed on quiescent breast cancer cells and may be used therapeutically to override tumor grown by stromal breast tissue stem cells.

keywords: Collagen-VII, Mesenchymal stem cells, Cancer-associated fibroblasts, Dormancy, Breast cancer.

62825442008

EFFECTS OF THE TRANSPLANTATION OF MESENCHYMAL STEM CELLS ON THE PATHOMORPHOLOGICAL VARIABILITY IN THE COURSE OF EXPERIMENTAL INFLUENZA PNEUMONIA

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Introduction
In modern medicine, the transplantation of mesenchymal stem cells (MSC) is utilized as a universal system for organism's protection and renewal under normal and pathological conditions. However, there are only a few studies concerning the use of mesenchymal stem cells to treat respiratory viral infections. This study aimed to estimate the morphological direction of MSC action in lung tissues of the experimental animals infected with influenza A(H1N1).

Methodology
Forty non-inbred mice (14-18 g) were used in experiments. An influenza virus strain A/FM/1/47/H1N1 was used to develop an experimental model. The strain was adapted to infect the lungs of white mice. Primary cultures of MSC from Wharton jelly were isolated from umbilical cords after obtaining informed consent. All animals were separated into eight groups (including the control group), depending on the concentration and route of administration (intravenous or intranasal) of MSC preparations. We performed pathohistological and morphometric studies of lung tissues for comparative morphological characterization of the effects of MSCs administration in experimental influenza virus-induced pneumonia.

Results
Transplantation of MSCs in experimental animals infected with the influenza A(H1N1) virus stimulated the body's immune system and led to the development of a powerful local cell-mediated reaction in the lungs. A pronounced cellular lymphoid-macrophage response developed in the foci of viral pneumonia and the lungs respiratory sections with preserved ventilation on the third day of the disease.
Subsequently, the processes of cell infiltration in the foci of serous-hemorrhagic viral pneumonia progressed. Cellular infiltration was observed mainly in the interalveolar septa, the lumen of the alveoli, bronchioles, and bronchi. The morphometric indicators of the prevalence and intensity of hyperplasia in broncho-associated lymphoid tissue (2.42±0.26), infiltration by lymphoid cells of the connective tissue of the lungs (2.75±0.31), the presence of lymphoid cells in the alveoli (4.00±0.65) exceed the indicators of the control group by 2.42; 2.07 and 3.01 times, respectively ($p<0.05$) regardless of the concentration and method of administration of MSCs into infected animals.

Conclusion
Thus, the therapeutic potential of MSCs in animals infected with the influenza A (H1N1) virus was aimed at stopping the development of acute diffuse exudative serous hemorrhagic inflammation of the lungs. At the same time, the form of inflammation changes. A pronounced diffuse proliferation of cells of broncho-associated lymphoid tissue was the main morphological feature of the inflammatory process.

keywords: influenza virus, influenza pneumonia, mesenchymal stem cells

73296321255

EXAMINING BIOLOGICAL PROPERTIES OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/ STROMAL CELLS OBTAINED FROM HEALTHY AND DIABETIC DONORS

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Introduction Mesenchymal stem/ stromal cells (MSCs) represent one of the most extensively investigated adult stem cell populations due to their pro-regenerative activity observed following their application into injured tissues. Diabetes mellitus type 2 (T2D) is a serious civilization disease occurring frequently in western societies. The prolonged high glucose level in blood may cause damage to many organs, including bone and joint disorders e.g., osteoarthritis. It has been shown that the physiological and clinical status of human cell donors, as well as the type of source material for MSCs isolation, could affect biological properties of these cells. Thus, this study aimed to examine the biological properties of adipose tissue-derived MSCs (AT-MSCs) harvested from healthy and T2D donors.

Methodology Human AT-MSCs obtained from healthy donors and patients suffering from T2D were purchased from Lonza and underwent comparable biological features characterisation including assessment of: morphology, viability in various conditions, and antigenic profile, proliferation rate as well as trilineage differentiation potential in vitro. In our study, three types of expansion cell culture media (CM) were used: i) control – standard CM, ii) standard CM containing high glucose concentration (to mimic diabetic conditions), or iii) standard CM containing high glucose concentration and addition of insulin (to mimic insulin-treated diabetic conditions). Appropriate media (StemPro Differentiation Kits) were used to stimulate cell differentiation into adipocytes, chondrocytes, and osteocytes and were supplemented with high glucose concentration or high glucose concentration and addition of insulin accordingly to the initial expansion conditions.

Results In our preliminary study, we observed no significant differences in morphology, viability, proliferation rate as well as expression of positive (CD90, CD105) and negative (CD19, CD45) MSCs markers between AT-MSCs derived from healthy and T2D donors in the tested media. In the case of osteogenic differentiation, we also did not observe any significant differences in expression of genes related to this differentiation pathway at different time points (3, 7, 14, or 21 days of differentiation) between the groups, which was confirmed by Anilazine Red histochemical staining. However, AT-MSCs derived from T2D donors exhibited a significant decrease in adipogenic genes expression along with decreased aggregation of lipid droplets observed after histochemical staining, when compared to healthy donors. In case of chondrogenic differentiation, we observed a statistically significant impact of glucose or glucose and insulin supplementation on expression of selected genes activated during chondrogenesis, which indicates important role of culture conditions on healthy and diabetic MSCs.

Conclusions To summarise, AT-MSCs obtained from healthy and T2D donors exhibited similar biological properties including proliferation, viability, morphological and antigenic stability in the tested conditions as well as mesodermal differentiation capacity in vitro, and as such may be considered for future use in autologous applications in human patients. However, the

appropriate concentration of glucose or glucose and insulin in the expansion medium may affect the expression of some genes critical for differentiation of AT-MSCs, which requires future optimization, especially for clinical applications.

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keywords: mesenchymal stem cell, human adipose tissue-derived MSCs, Diabetes mellitus, biological properties

31412757366

HUMAN MESENCHYMAL STROMAL CELLS ISOLATED FROM BONE MARROW AND FROM WHARTON'S JELLY DIFFER IN RESPONSE TO HYPOXIA MIMICKING SELECTIVE HIF PROLYL HYDROXYLASE 2 INHIBITOR

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Mesenchymal stromal cells (MSCs) possess immunomodulatory abilities that make them a promising candidate for the treatment of immune-mediated diseases, particularly those with an autoimmune component. We have recently shown that preconditioning human bone marrow (BM) MSCs with Vadadustat – hypoxia mimicking agent, may constitute a valuable approach to improve the therapeutic properties of MSCs. Vadadustat is one of hypoxia inducible factor prolyl hydroxylase (HIF-PH) inhibitors, known also as HIF stabilizers. MSCs isolated from Wharton's jelly (WJ) constitute an attractive alternative to BM-MSCs. Perinatal tissues are available in unlimited amounts, and WJ-MSCs have a greater proliferative activity than BM-MSC with comparable immunomodulatory potential. Therefore, the objective of this study was to compare the effect of HIF-PH inhibitor on these two cell types. Moreover, we aimed to evaluate if HIF stabilizers affects the interaction between MSCs and lymphocytes in terms of T regulatory (Tregs) cells formation. First, we have assessed the effect of two different HIF-PH inhibitors on the HIF1α expression. Western blot analysis revealed that IOX4 (selective HIF prolyl-hydroxylase 2 inhibitor) used in concentration 5 µM caused substantially stronger HIF1α stabilization in both BM-MSCs and WJ-MSCs than Vadadustat in concentration 40 µM. Moreover, the effect induced by IOX4 lasted at least 48h whereas the effect of Vadadustat was not longer than 24h. The effect of IOX4 on viability and proliferative activity of BM-MSCs and WJ-MSCs was assessed using MTT assay and BrdU assay, respectively. In both tests WJ-MSCs appeared to be significantly more sensitive to IOX4 treatment than BM-MSCs. In comparison to control (solvent only), the mean viability of BM-MSCs and WJ-MSCs after 48h treatment with IOX4 decreased by 5% and 18%, respectively (the difference in response between two cell types – $p=0.02$). Analogous decrease in BrdU assay amounted 5% and 52% for BM-MSCs and WJ-MSCs, respectively (the difference in response between two cell types – $p=0.03$). Next we evaluated the effect of both analyzed MSC types on Treg formation in the population of allo-activated lymphocytes. Both BM-MSCs and WJ-MSCs significantly increased the proportion of Tregs (defined as CD4+CD25++CD127-) in CD3+CD4+ cells after 3 days of co-culture of allo-activated lymphocytes with MSCs. An average increase in Tregs proportion in the presence of BM-MSCs and WJ-MSCs amounted 63% and 43%, respectively ($p<0.05$ for both MSC types in comparison to allo-activated lymphocytes without MSCs). Pretreatment of MSC with IOX4 before co-culture with lymphocytes did not significantly affect the effects of MSCs on Tregs formation regardless of MSC type used.

To summarize, IOX4, selective HIF prolyl hydroxylase 2 inhibitor has cytotoxic effect on hWJ-MSCs, which appeared to be significantly stronger than on hBM-MSCs. This difference in response may be due to the various physiological conditions of the niches from which both cell

types are derived. These results suggest that mimicking hypoxia may have different effects on MSCs from various sources. Nevertheless, our results indicate that these differences do not necessarily translate into the effects of complex interactions of MSCs with immune cells.

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keywords: mesenchymal stromal cells, bone marrow, Wharton's jelly, hipoxia inducible factor, prolyl hydroxylase inhibitor, T regulatory cells

41883668648

INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS (IMSC) AS A POWERFUL CELL SOURCE FOR CELL-BASED THERAPIES

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Introduction
Mesenchymal stem cells (MSCs) are self-renewing, non-specialized, multipotent cells with great potential to differentiate into various tissue cells. Due to their secretion activity, migration, adhesion and homing abilities, MSCs are currently highly investigated stem cells and the one most commonly used in regenerative medicine. The use of adult MSC is still challenging, since a limited number of these stem cells can be obtained from a single donor and a substantial cell quantity is required for their clinical application. Other challenges include the limited adult MSC proliferation capacity, which may significantly constrain their capabilities of immunomodulation and secretion of bioactive factors. Considering these facts, we differentiated induced pluripotent stem cells (iPS cells) towards mesenchymal stem cells (iMSCs) using the optimized xeno-free culturing protocol. Next, we have compared properties of obtained cells with MSCs isolated from Wharton's Jelly (WJ-MSCs).

Methodology
We used two protocols based on differentiating medium supplemented with FBS and human platelet lysate respectively. We examined phenotype and properties of acquired iMSCs in terms of their: morphological features (microscopy techniques), expression surface markers (flow cytometry), trilineage differentiation potential, expression of selected factors (RT-PCR) and cell proliferation by use of BrdU assay (flow cytometry). We have also evaluated cellular senescent during long time differentiating process by senescence-associated β-galactosidase (SA-β-gal) activity assay.

Results
IMSCs we obtained show the minimal criteria outlined for MSCs by the International Society of Cellular Therapy (ISCT). The cells adhere to plastic in standard culture conditions and display fibroblast-like morphology and ability to trilineage differentiation towards adipogenic, osteogenic and chondrogenic lineages. Cytometric analysis revealed high expression of specific mesenchymal markers. More than 90% of cells were CD73, CD90, CD105 and CD146 (MSC progenitor marker) positive, whereas they do not express hematopoietic antigens (CD45, CD14, CD19, CD34, CD3). During long term expansion iMSCs show high proliferation potential with insignificant number of SA-β-gal positive cells. The purity of populations has been assessed by reduced expression of OCT-4, SOX-2, Nanog and EpCAM compared to the population of iPS cells. It should be noted that iMSCs injected into mice did not form tumors.

Conclusions
The relatively easy process of obtaining iMSCs make them an inexhaustible source of MSCs that could be used for clinical applications. Since iMSCs exhibit similar properties as MSCs isolated from natural tissues, they open the wide possibilities of their use as off-the-shelf allogeneic cell-based therapeutics.

This work was supported by research grant 2018/29/B/NZ5/00915 from the National Science Center in Poland.

keywords: iPS cells, induced mesenchymal stem cells, cellular therapy

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INFLUENCE OF MACROMOLECULAR CROWDING ON EXTRACELLULAR MATRIX DEPOSITION AND MESENCHYMAL STEM CELLS

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In cell-based therapies, cells are removed from their crowded physiological context and expanded in a dilute culture medium. In this way, the enzymatic reactions involved in the conversion of pro-collagen to collagen are slowed, leading to a decrease in the deposition of extracellular matric (ECM). [1,2] Macromolecular crowding (MMC), is a biophysical approach that can be used to imitates the dense extracellular space, accelerating the deposition of the ECM in vitro and mimicking the in vivo environment. [3,4]
Although, MMC has been shown consistently to enhance and accelerate ECM deposition, the ideal crowding agent is still elusive. Thus, we assessed the potential of carrageenan, a sulfated polysaccharides and Ficoll™ 70 kDa and Ficoll™ 400 kDa cocktail, a neutral polysaccharides as MMC agents.
The crowders effect in vitro was evaluated in human Bone Marrow Derived Stem Cells (hBMSC) after 5, 8 and 11 days of cells culture. A non crowding condition was used as a control group. The crowders did not affect cell morphology and did not compromise cell viability over the assessed timepoints. Even though, cell metabolic activity using alamarBlue® assay showed a decrease in cell metabolic activity when cells were cultured with carrageenan and Ficoll™ 70 kDa and Ficoll™ 400 kDa cocktail compare to the control group.
SDS-PAGE and corresponding densitometry analysis showed that in all the timepoints both the crowders enhanced the deposition of collagen type I compare to the control group. However, carrageenan induced higher collagen type I deposition compare to the control group and Ficoll™ 70 kDa and Ficoll™ 400 kDa cocktail in all the timepoints. Immunocytochemistry and complementary relative fluorescence intensity analysis for collagen type I and collagen type III showed that both the crowders exibited collagen type I and collagen type III deposition in the timepoints assessed.
The data suggested the importance of the MMC in cell culture and for possible tissue engineering applications and the potential of carrageenan as macromolecolar crowders agents. Further analysis will be conducted to analyse the effect of MMC on the genome and proteome in hBMSC.
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keywords: Macromolecular crowding, stem cells, ECM deposition

73296303577

MESENCHYMAL STEM CELLS TO PREVENT OR TREAT GRAFT VERSUS HOST DISEASE IN HEMATOPOIETIC CELL TRANSPLANTATION: A SYSTEMATIC REVIEW

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Introduction
Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for malignant blood diseases. However, long-term results are limited by the high morbidity and mortality associated with the development of graft versus host disease (GVHD). The use of mesenchymal stem cells (MSCs) has emerged as a safe and effective therapeutic option for GVHD due to its immunomodulatory potential.

Aim
In this review we discuss the use of MSCs for the prevention or treatment of GVHD in HSCT and their efficacy and safety in these clinical applications.

Methodology
Using PubMed, Scopus and Web Of Science databases and applying the search algorithm: (“Mesenchymal Stem Cells” OR “Mesenchymal Stromal Cells” OR “Bone Marrow Mesenchymal Stem Cells” OR “MSC”) AND (“Graft vs Host Disease” OR “Graft-Versus-Host Disease” OR “Disease, Graft-Versus-Host” OR “Graft Versus Host Disease” OR GVHD), 103 international scientific publications were found that correspond to clinical studies, among which 75were included in this review.

Results
We included in this review 24 articles related to the use of MSCs as prophylaxis and 51 articles related to their use as treatment for GVHD. In them we identified that the main MSCs sources are: bone marrow, adipose tissue, cord blood and placenta. The route of administration is intravenous infusions and the dose is 1x106cells/kg.
It is known that MSCs are characterized by their low immunogenicity due to their low expression levels of major histocompatibility complex I (MHC), no expression of MHC II and costimulatory molecules, such as CD80, CD86, and CD40, therefore they are able to evade allogeneic rejection. Increasing evidence has emerged suggesting that these cells possess the abilities to secretea variety of cytokines that may create a more favorable bone marrow microenvironment, improvingdonor engraftment by supporting the hematopoiesis. Besides MSCs can modulate immune responses and reduce the risk of GVHD through the inhibition of the activation and proliferation of T and B lymphocytes and the downregulation of inflammatory cytokine expression such as TNF-α, IL2R-α, elafin and INF-γ.

Conclusion
In conclusion, the immunomodulatory properties of MSCs after and during allogeneic HSCT are very useful in the clinical setting to prevent and treat GVHD.

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keywords: Mesenchymal stem cells (MSC), graft-versus-host disease (GHVD), hematopoietic stem cell transplantation (HSCT) and cell therapy

83767252119

MESENCHYMAL STROMAL CELLS DERIVED SECRETOME PROTECTS BRAIN TISSUE FROM TRAUMA: EVIDENCE FROM A NEWLY DEVELOPED IN VITRO MODEL

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Mesenchymal stromal cells (MSCs) have shown great promise in experimental traumatic brain injury (TBI), conferring protection mainly through the release of bioactive factors (secretome). In vitro models, mimicking the in vivo condition in a simpler but representative environment may be fundamental to investigate secretome mechanisms of action. Nonetheless, TBI research lacks relevant 3D in vitro models that can serve as a bridging approach between cell culture and in vivo experiments. The aim of this study was to develop an in vitro model of brain contusion on organotypic brain slices and to test the efficacy of MSC-secretome obtained from human umbilical cord perivascular tissues.

Organotypic brain slices from the prefrontal cortex of newborn mice were subjected to controlled biomechanical impact (CCI) by an electromagnetic device. Compared to the control (CTRL) condition, CCI slices showed a time-dependent increase in cell death evaluated by lactate dehydrogenase (LDH) and propidium iodide incorporation (PI) assays. More in-depth, regional specific analysis of PI showed the highest values at the lesion core, and a gradual decrease moving away from it. An overtime increase in neuronal damage was revealed by neurofilament light (NFL) biomarker released in culture medium. Gene expression analysis 48h after injury showed significant downregulation of neuronal (MAP2) and upregulation of pan glial markers (CD11b and GFAP) after CCI.

MSC-secretome delivered 1h post-injury reduced PI values at the lesion core of CCI slices and reduced neuronal damage, as revealed by the reduction of NFL release in the medium and by a rescue effect of CCI-induced downregulation of both mRNA MAP-2 and BDNF.
In conclusion, we established a reliable in vitro model that recapitulates key features of TBI pathology and provided evidence for the protective efficacy of MSC-secretome.

keywords: TBI/in vitro/organotypic slices/MSCs/Secretome

94238118324

OXIDATIVE STRESS RESPONSE IN ADIPOSE-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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Introduction
Adipose tissue-derived mesenchymal stem/stromal cells (adMSC) are shown to support wound healing due to their regenerative and immunomodulatory capacities. However, cell survival in the specific wound microenvironment might be affected by oxidative stress, so their ability to regenerate tissues may be impaired. In this study, we investigated the effects of oxidative stress induced by repeated treatment with hydrogen peroxide (H2O2, only briefly stable in medium) or the enzyme glucose oxidase (GOX, which catalyzes the long-term production of H2O2). Using this cell culture model, the short- and long-term effects of oxidative stress on cell survival, proliferation, migration, and differentiation ability were examined.

Methodology
adMSC were isolated from adipose tissue by a CD34-specific isolation protocol. adMSC in passage 4 were exposed to different concentrations of H2O2 or GOX and the viability, proliferation, migration (scratch assay), adipogenic, and osteogenic differentiation capacity of adMSC were examined. At the same time, the extent of oxidative stress was quantified by Amplex Red reagent (detects H2O2) and DCFDA (detects reactive oxygen species/ROS). With this approach, we aimed to relate the induced oxidative stress to the cellular response.

Results
First, we detected the toxicity threshold of the two different oxidative stressors. Only non-toxic concentrations were used for all further investigations. In addition, no significant influence on the metabolic activity of the adMSC could be shown in the amounts of non-toxic H2O2 and GOX used. The adipogenic differentiation capacity (examined over a period of 14 days) was not changed by the non-toxic concentrations of H2O2 or GOX. The degree of osteogenic differentiation (examined over a period of 35 days) was under the influence of higher oxidative stress (0.5 mM and 1 mM H2O2, respectively 5 mU/ml and 10 mU/ml GOX) due to an increase in extracellular calcium deposition. Initial investigations show that the migration activity (detected using the scratch assay) is significantly increased by the addition of non-toxic amounts of H2O2 and GOX.

Conclusion
These results indicate a certain resilience of adMSC to oxidative stress. We could show that osteogenic differentiation and migration ability are increased by non-toxic concentrations of H2O2 and GOX. We are currently investigating how oxidative stress affects the inflammatory response of cells. For this purpose, cytokines (e.g. IL-6) and growth factors (e.g. TGF-β, bFGF) important for wound healing are examined. These fundamental investigations can help to describe the therapeutic potential of adMSC in the wound healing process more precisely and, if necessary, to influence them.

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keywords: Mesenchymal stem/stromal cells, hydrogen peroxide, glucose oxidase, oxidative stress, wound healing

83767240806

PRODUCTION OF BIOACTIVE AGENTS UNDER VARIOUS CULTURE CONDITIONS TO ADJUST THE COMPOSITION OF THE FACTORS IN A CONDITIONED MEDIUM FOR REGENERATIVE MEDICINE

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Introduction: Numerous clinical trials in which MSC cells were used have lead to different results, not always as positive as expected. According to our hypothesis MSC can be replaced in some therapies by active factors they produce. Therefore, we decided to replace the primary MSC cultures with immortalized line derived from human adipose tissue (HATMSC1) to produce biologically active factors for basic and application study.

Methodology: The MSCs phenotype of immortalized cell line HATMSC1was confirmed using the flow cytometry method. The content of biologically active factors in conditioned media (CM) were assessed in standard culture conditions (however, without fetal bovine serum) and in CM stimulated by hypoxia and immunomodulatory factors (TNF-α or INF-γ or LPS) using RayBio® C-Series Human Cytokine Antibody Array C1000. The level of five selected cytokines was confirmed using the Multiplex ELISA method, Human Cytokine/Chemokine Magnetic Bead Panel MILLIPLEX® MAP Kit. Pro-proliferative activity of CM was evaluated using MTT assay and target cell lines involved in wound healing: fibroblasts (MSU-1.1), keratinocytes (HaCaT) and endothelial cells (HSkMEC.2). Pro-angiogenic activity was evaluated using Capillary-like Structures Formation Assay in the Matrigel.

Results: Rapidly proliferating immortalized cell line (HATMSC1) with the phenotype of MSCs produced above 100 out of 120 analyzed cytokines. Hypoxia trigger augmentation in the production of certain pro-angiogenic growth factors e.g. Angiogenin, IL-6, MCP-1, GRO, IL-8, TPO and VEGF by 20% - 30%. The production level of selected factors as assessed using the Multiplex ELISA was highest in the case of IL-8 and VEGF-A and reached a value of 2–2.5μg/mL while angiogenin was produced at the lowest concentration of 250 pg/mL.
The immunomodulatory factors like TNF-α, INF-γ or LPS caused an increase in production of many bioactive factors such as: GM-CSF, IL-5, IL-6, MCP-1, RANTES or IL-8 from 100 to 1200%!, compared to CM from standard culture conditions. Interestingly, the concentration of cytokines depends also on the cell density at the time of setting up the culture. There were cytokines produced more efficiently in dense culture (MCP-1 or IL-8) and those that were produced more efficiently in culture with low density (IL-6 or Osteoprotegerin). The in vitro pro-proliferative and pro-angiogenic activity of the CM produced by the HATMSC1 line was confirmed. Proliferation of target skin-origin cell lines increased up to 300% of control after 72h of culture when 50% CM was used. The highest pro-angiogenic effect was observed by increasing average mesh size by 200% over the control and was achieved after 14h with the use of 10% CM.

Conclusion(s): The results showed that immortalized cell line HATMSC1 cultured in the

presence of TNF-α or INF-γ or LPS increased their ability to produce biologically active factors in terms of both their quantity and concentration. The data suggest that the factors produced under different culture conditions could potentially be used in different clinical conditions in regenerative medicine e.g. when increased concentration of pro-angiogenic factors is needed or in inflammatory diseases when high concentration of immunomodulatory factors is desired.

keywords: immortalised cell line, growth factor production, regenerative medicine,

52354530846

THE EFFECT OF INTERFERON-Γ AND INTERFERON-Α PRE-CONDITIONED MESENCHYMAL STEM CELLS TRANSPLANTATION ON INFLUENZA VIRUS INFECTION ON MURINE MODEL

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Introduction: The enhancement of mesenchymal stem cells (MSC) therapeutic potential is considered an important scientific task. Though it was demonstrated, that pre-conditioning with interferon-γ (IFNγ) is able to enhance the MSC immunomodulatory properties, no current data is known from literature about the effects of interferon-α (IFNα) on MSC. The aim of the present study was to evaluate the therapeutic effect of interferon-γ (IFNγ) and interferon-α (IFNα) primed Wharton jelly MSC.

Methodology: The experimental model of influenza pneumonia was obtained by intranasal infection of white non-inbred mice, with the influenza virus A/FM/1/47/H1N1/, adapted for mouse lung tissue, with the infection titer 4,0 lg ID50, and hemagglutinin titer 1:256 HA units/0,2 ml. Human Wharton jelly MSC at passage 2 were fed with fresh culture media containing recombinant human IFNγ (20 and 500 U) and recombinant human IFNα (2000 U and 10000 U), overnight. After that, MSC were collected, and transplanted to model mice intravenously, at 2x105 per mouse.

Results: MSC transplantation enhanced the survival comparing to non-treated control. The increase of survival rate in groups, treated with IFNγ-primed MSC reached 80% (both 20 and 500 U), while mice, treated with the IFNα-pre-conditioned MSC (both 2000 and 10000 U), showed 20% increase in survival level. Histological analysis demonstrated an early formation of dence peribronchial and perivascular lymphoid macrophage infiltrates in lung parenchyma, which are the morphological evidence of easier experimental viral pneumonia progression. These formations have the high level of specialization of local cellular immunity and match all the criteria of the first line of antiviral defense.

Conclusion: The present work showed, that pre-conditioning influences the therapeutic effect of MSC on viral infections. The demonstrated effects need further investigation.

keywords: mesenchymal stem cells, interferon-γ , interferon-α, influenza virus, influenza pneumonia

83767245288

THE IMPACT OF GRAPHENE-BASED SUBSTRATES ON BIOLOGICAL AND FUNCTIONAL PROPERTIES OF HUMAN MESENCHYMAL STEM CELLS - SIGNIFICANCE FOR CARDIOVASCULAR REPAIR

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Introduction
Cardiovascular disease (CVD) is a general term for a group of disorders of the heart and blood vessels. According to statistical data of World Health Organization (WHO), CVD is one of the most prevalent disease and the leading cause of death globally in recent years. Therefore, it is justified to search for new, effective therapeutic methods such as stem cell therapy and tissue engineering. One of the most promising groups of stem cells are mesenchymal stem cells (MSCs). MSCs are multipotent stem cells characterized with high proliferative potential, paracrine activity and the ability to differentiate into many cell types. In order to effectively use the potential of MSCs for tissue regeneration, new biocompatible scaffolds for cell culture are still being sought for. An interesting material for biomedical applications due to their unique physicochemical properties are graphene oxide (GO) and reduced graphene oxide (rGO).

Methodology
The main goal of this study was to investigate the potential of GO and rGO substrates as scaffolds that can promote cardiomyogenic and angiogenic differentiation of MSCs in in vitro and in vivo studies.
Different types of solvents (water, ethanol), size of flakes, level of GO reduction and graphene layer thickness have been tested to obtain the most promising cell culture surfaces. The influence of the graphene-based scaffolds on the biological and functional properties of MSCs, such as morphology, viability, proliferative capacity, adhesion ability were investigated. The cytotoxicity of the graphene substrates was evaluated using Cytotoxicity Detection Kit (Sigma Aldrich). Based on the collected results, the substrates with the best biocompatibility were selected. Next, the chosen biomaterials were used to differentiate MSCs into heart tissue and blood vessel cells. In order to investigate the efficiency of the differentiation of MSCs, an analysis of gene expression was performed. Moreover, the effectiveness of angiogenic differentiation of MSCs on selected rGO surfaces was evaluated in murine model of hind limb ischemia.

Results
Obtained results showed that the biocompatibility of the tested substrates depends on the

thickness of the graphene layer, the size of the flakes and the level of graphene reduction. The most appropriate graphene-based surfaces for MSCs culture were large flakes of GO and slightly reduced rGO. It was observed that in MSCs cultured on one type of slightly reduced rGO surface the expression of endothelial genes increased in comparison to the control conditions.

Conclusions
The conducted research indicates that graphene-based substrates, can have a different biological effect on MSCs, depending on the thickness and size of graphene flakes as well as the level of GO reduction. Moreover, selected rGO surfaces may promote angiogenic differentiation of MSCs, which could contribute to their future use in cardiovascular repair. However, further studies are required to analyze these phenomenon.

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keywords: mesenchymal stem cells, graphene, scaffolds, tissue engineering, regenerative medicine

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THE INFLUENCE OF HUMAN MESENCHYMAL STEM CELLS (HMSC) SECRETOME ON EPILEPTIC MICE-DERIVED ORGANOTYPIC HIPPOCAMPAL CULTURES (OHC)

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Introduction:
One of the most common labor disturbances is the reduction of prenatal blood flow which results in reduction of oxygen level in neonatal brain. This in turn can lead in long term to the development of drug-resistant epilepsy. Main therapy in the case of epilepsy is administration of sedative drugs which alleviate degree of seizures but also decline the child's cognitive development. Hence, it is an urgent problem to develop a novel therapy that would make the neonatal seizures less intensified with possibility of correct development. Stem cell-based therapy has attracted much attention because of not only its regenerative property but also its long therapeutic time window. The study determines how the secretome of human Mesenchymal Stem Cells (hMSCs) may influence Organotypic Hippocampal Cultures (OHC) derived from NOD SCID mice with Temporal Lobe Epilepsy (TLE) induced using pilocarpine.

Methodology:
Experiments were performed by culturing OHC in MSCs – conditioned media, which was prepared by incubating MSCs in OHC-dedicated medium for 48 hours. MSCs secretome influence on pilocarpine-induced hippocampal tissue damage was carried out by the assessment of OHC morphology, followed by colorimetric cell viability and mortality tests and also the measurement of chosen pro-inflammatory factors in the culture medium and/or OHC lysates.

Results:
Our data report indicates that the MSCs cell therapy is effective in preclinical studies in terms of viability and morphology of OHC amelioration, as well as improvement of immune stage of damaged brain tissue.

Conclusions
We have proven that the pilocarpine mice derived OHC are a viable model to investigate the efficacy of stem cell-based therapies. Easy obtainability of MSCs, combined with no ethical problems in cell collection, no tumorigenicity, low immunogenicity and easy preparation seem to be crucial when translating into the clinic.

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keywords: human Mesenchymal Stem Cells, epilepsy, regenerative medicine, Organotypic Hippocampal Cultures

94238173048

VOLUMETRIC MASS DENSITY OF MESENCHYMAL STEM CELLS - A NEW METHOD FOR THE DETERMINATION OF AN ESSENTIAL PARAMETER

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Mesenchymal stem/stromal cells (MSC) have shown their regenerative potential in vitro, in preclinical models and are increasingly used in clinical applications as well. For cell therapeutic applications, MSC must be isolated from their resident tissues. For the isolation of MSC from adipose tissue, more and more systems are entering the medical device market. During the automated or semi automated isolation procedures within these systems the cells are subjected to mechanical strain. In order to design devices that isolate intact, viable cells, optimizations with experimental methods or fluid flow simulations become necessary. These methods require the input of basic parameters like cell volumetric mass density. The determination of this parameter is not trivial and currently avalable methods are very elaborate, cost intensive and experimental and thus not readily available. With the introduced method we describe a user-friendly method for the determination of the volumetric mass density of cells like adMSC with a density meter and a cell counter. The method is based on the hypothesis of a linear relationship between the volumetric mass density of the cell suspension and the volumetric mass density, number and diameter of the cells in a cell suspension. The volumetric mass density of the cell suspension and its medium without any cells are measured. Cell number and cell diameter distribution are determined using a cell counter. From these values the volumetric mass density of the cells can be determined. The values calculated with the described method was compared to volumetric mass density evaluated using the standard method of density centrifugation. For cultured adMSC the determined a median volumetric mass density of 1,0525 g/cm3 using the proposed method and 1.045 g/cm3 using density centrifugation. Thus, both methods show only a 0,7% difference. The main advantage of our newly introduced method is the shorter time required to perform the measurements, the lower amount of cellular material needed in the process. Furthermore, the devices used are readily available on the market and thus easily accessible.

keywords: Volumetric mass density, cell suspension density, MSC, adMSC, density meter

PS42
Microphysiological models as powerful preclinical tools

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A UNIQUE MULTI-ORGAN IN VITRO MODEL FOR PERFORMING MORE PREDICTIVE PRECLINICAL TOXO-EFFICACY ASSAYS

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INTRODUCTION: Many human disorders display unknown tissues relationships affecting disease progression and drugs response (1). While in vitro assays fail in recapitulating organ-organ connection, animal models reveal species-specific drug outcomes. To this purpose, a novel multi-organ microphysiological system (MIVO®) has been adopted to fluidically connect 3D ovarian cancer tissues with a hepatic cellular model and simulate the systemic cisplatin administration for investigating its anticancer efficacy and simultaneously measuring potential hepatotoxic effects.
METHODS: Human hepatocellular (Hep-G2) and ovarian cancer (SKOV-3) cells line have been used to realize liver models and 2D/3D tumor model (2). Computational fluid-dynamic modeling has been performed to simulate the capillary blood velocity, that was set-up within MIVO® where 3D ovarian cancer and the liver model were cultured fluidically connected. First, a drug concentration (10-100 µM) screening was performed by using 2D and 3D single organ models. Then, drug efficacy and toxicity assays were assessed in MIVO and compared with both static co-cultures and single organ models. Ovarian and liver cells death, half maximal effective concentration (EC50) and median lethal dose (LD50) for Skov-3 cells and Hep-G2 cells were quantitatively assessed. Cisplatin effects were also qualitatively by immunofluorescence.
RESULTS: A linear decay of Hep-G2 and Skov-3 cells viability was observed with increasing cisplatin concentration after 48h of treatment. Furthermore, the 3D ovarian cancer model demonstrated higher drug resistance than the 2D model. Finally, reduced efficacy against the 3D ovarian cancer tissue and hepatotoxicity were observed in the MIVO compared with single organ model (0.5X of EC50 and 2X of LD50).
DISCUSSION AND CONCLUSIONS: Results highlight that the introduction of 3D cells culture and multi-organ fluidic connections resembling the in vivo conditions significantly impact in both drug efficacy and toxicity outcomes, indicating the importance of developing more predictive pre-clinical tools for drug screening.
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keywords: multi-organ model, toxicity, efficacy, microphysiological system

83767201604

BLADDER CANCER CELLS INFLUENCE HUMAN ADIPOSE-DERIVED STEM CELLS CHARACTERISTICS IN VITRO

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Introduction
Stem cell-based therapies are considered one of the most promising disciplines in the field of biomedicine. Bladder cancer patients could benefit from such therapies directed to promote healing after invasive surgeries or to lessen urinary incontinence, a common side effect of both cancer itself and the treatment. One particularly attractive source of cells for bladder regeneration is adipose tissue. Adipose-derived stem cells (ASCs) of urological patients show similar characteristics, e.g. growth rate, surface markers expression, and differentiation potential to ASCs isolated from healthy individuals. They also secrete a wide variety of soluble mediators that promote tissue regeneration. However, local delivery of ASCs producing large amounts of paracrine factors may promote tumor reactivation.

Methodology
To characterize the interplay between ASCs and bladder cancer cells we used an in-direct co-culture system. ASC52telo mesenchymal stem cells immortalized with hTERT (SCRC-4000) were purchased from ATCC. These primary cells undergo quality control tests to guarantee cell identity. According to International Society for Cell & Gene Therapy (ISCT) criteria ASC52telo cells show high expression of CD29, CD44, CD73, CD90, CD105, CD166 (>90%) and low expression of CD14, CD19, CD34, CD45 (<5%). They retain a multipotent phenotype with the ability to differentiate into adipocytes, osteoblasts. ASCs were seeded on multiwell cell culture plates and bladder cancer cells (5637, HT-1376, and HB-CLS-1) on PET inserts. After 72h co-culture, we analysed morphology, immunophenotype, growth, viability, migration, and activation of intracellular signaling pathways.

Results
Cancer cell-derived factors altered ASCs morphology. Cells with atypical shape and significantly enlarged volume appeared within the monolayer. These morphological changes were not, however, accompanied by changes in the cell surface markers expression. Incubation in conditioned medium (CM) containing soluble mediators secreted by 5637 and HB-CLS-1 cells decreased ASCs number. A significant reduction in viability and proliferation was also noted. In turn, no significant differences were observed when ASCs were co-cultured with HT-1376 cells, which indicates that induced effects may be cell-line dependent.

Conclusions
Adipose-derived stem cells represent one of the most promising cell populations for regenerative medicine. They can be easily harvested in larger quantities and share many of the same regenerative properties as other mesenchymal stem cells. As urological cancer patients are considered good candidates for cell-based therapies, in vitro-expanded ASCs could be used

to promote healing after invasive procedures or for organ remodeling surgeries. However, reconstructive cellular therapies for patients with cancer history may promote tumor growth. Our findings indicate that soluble mediators secreted by non-muscle-invasive urinary bladder cancer cells influence ASCs characteristics. For that reason, comprehensive knowledge of the crosstalk between tumor and stem cells seems essential to assess the potential risks of ASC-based regenerative therapies after cancer surgery.

keywords: bladder cancer, adipose-derived stem cells, cell-based therapies, cancer recurrence

52354525977

FORMATION AND LONG-TERM CULTIVATION OF RETINAL ORGANOIDS IN MICROFLUIDIC SYSTEMS

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INTRODUCTION
Advances in organoid research has led to the development of retinal organoids capable of forming optic cups as well as giving rise to all major cell types of the neuroretina, most notably mature photoreceptors. These retinal organoids can be used for studying retinal development or to serve as a source of photoreceptors for transplantation therapy. However, several challenges remain, such as heterogeneity between organoids, poor photoreceptor maturation and the degradation of retinal ganglion cells in long term cultures. Microfluidic platforms have the potential to help overcome some of these challenges by allowing for automation and precise control of the microenvironment. For this work we designed a one-stop microfluidic device capable of retinal organoid formation and maturation to decrease labor intensity while improving organoid oxygen and nutrient supply.

METHODOLOGY
Microfluidic devices were fabricated from PDMS using a mold created with photo- and stereolithography. Retinal organoids were differentiated from human pluripotent stem cells inside a microfluidic device under continuous medium perfusion. The organoids were compared to a static control, grown in standard culture conditions, based on the analysis of morphology, cell viability, and cell differentiation.

RESULTS
Optimization of the shape and size of the microwells allowed for efficient formation of homogenous embryoid bodies within 24 hours after seeding. Subsequent cultivation under continuous medium perfusion led to the development of retinal organoids, as observed using live imaging. Retinal organoids cultivated within the chip developed at a timescale representative of the in vivo situation as it was evaluated by the expression of eye field transcription markers and retinal progenitor cell markers.

CONCLUSION
The proposed platform allows for standardized formation and long-term maturation of retinal organoids in a low shear-stress environment while decreasing labor intensity. An array of microwells allowed for quick aggregation and organoid formation. The device was capable of automated continuous medium perfusion to allow for retinal organoid cultivation and maturation without the need for transferring the organoids to a secondary culture vessel.

Acknowledgements
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keywords: organoids, retinal organoids, microfluidics, organ-on-chip

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MICRO-CONTACT PRINTING APPLICATIONS TO TEST CARDIAC TOXICITY ON HIPSC-CARDIOVASCULAR CELLS

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INTRODUCTION
Despite big efforts and capital invested, drug commercialization is severely hampered by high attrition rates impacting all development phases. Cardiac-side effects are the fourth cause of post-marketing drug withdrawal and stand behind almost 30% during the pre-marketing phase. With the blame on current testing systems, the generation of affordable and more representative cardiotoxicity testing platforms is a must to increase public confidence in drugs simultaneous to solid economical growth. Though the advent of human induced pluripotent stem cells (hiPSC) has promised to raise the prediction capacity of novel systems, the costs associated have highlighted the need to implement advanced technology towards decreasing it, whilst increasing throughput. Our aim is to use the micro-contact printing (µCP)-based “Single Cell Adhesion Dot Arrays” (SCADA) substrates1 with human cardiovascular phenotypes to develop a low-cost, high-throughput and easy-to-use system for human cardiotoxicity tracking.

METHODOLOGY
Micropatterned SCADAS were tuned to match the specific biological requirements of the hiPSC- cardiovascular phenotypes Hence, geometry, size and distribution of protein-patterns in combination with diverse cell density plating conditions are analyzed through optical detection by the use of conventional brightfield microscopy. We use small molecule-based differentiation protocols to obtain cardiomyocytes (hiPSC-CMs), smooth muscle cells (hiPSC-SMCs), endothelial cells (hiPSC-EC) and cardiac fibroblast (hiPSC-CFs). Once the best SCADA layout promoting single cell adhesion is selected, they are exposed to the cardiotoxic drug and cellular death monitored by optically assessing detachment.

RESULTS
A total of 5 different hiPSC lines were employed. The differentiation protocols generated highly-pure hiPSC-CMs, hiPSC-SMCs, hiPSC-ECs and hiPSC-CFs, as defined by FACS, IF and RT-qPCR for specific markers. For hiPSC-CMs, 45x25um Matrigel rectangular dots was the best SCADA design. 70-75% of initial dot array occupancy (DAO) is achieved, providing over a thousand cells (events) per SCADA that can be counted for high sensitivity. Collagen type I rectangular dots of bigger dimensions (90x30um) are employed to contain cells hiPSC-SMCs, hiPSC-ECs and hiPSC-CFs, rendering similar optimized conditions. Exposure to the cardiotoxic drugs doxorubicin or epirubicin at different concentrations elicited a drastic DAO decrease throughout the 96h of exposition, proving the capacity of the devised SCADAs to monitor toxicity; in addition, the different detachment kinetics are correlated to the IC50 values for the different lines, which

include hiPSCs derived from patients sensitive to doxorubicin. Moreover, these experiments show a higher sensibility of SMCs to the drug than other cardiovascular phenotypes. Finally, different phenotypes are included in a microfluidic chip in a first step towards building a multidimensional testing platform.

CONCLUSIONS
This work lays the foundations for a promising cardiotoxicity testing system, requiring low cell numbers, inexpensive materials and no specialized equipment. The system can uncover phenotype-specific drug sensitivity, and opens the way to high throughput analysis using microfluidics miniaturization, allowing the decrease of sampling volumes and cellular input.

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keywords: hiPSC-CMs; drug-cardiotoxicity; micro-contact printing

PS43
Multifunctional biomaterials supporting bone regeneration

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CERIUM, ZIRCONIUM AND COPPER DOPED ZINC OXIDE NANOPARTICLES FOR BONE REGENERATION AND ANGIOGENESIS

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Introduction
Bone tissue engineering (TE) is a therapeutic strategy to induce bone repair. Bone TE scaffolds often combine ceramic and polymeric materials to capture the advantages of each to create strong, tough and bioactive materials. However most current bone TE strategies still suffer from insufficient bone vessel recruitment into the defect region. Zinc Oxide has recently caught attention in the field of nanomaterials for its ability to induce upregulation of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) while improving the scaffold surface properties. In this research, we propose synthesis of novel composite materials made from polyurethane, hydroxyapatite and doped zinc oxide nanoparticles.

Methods
ZnO and doped ZnO nanoparticles (cerium, copper and zirconium doped nanoparticles) were synthesised using a hydrothermal flow system. The nanoparticles were characterised and the most viable doped ZnO nanoparticles were selected for scaffold synthesis. The freeze extraction salt leaching technique was used to fabricate porous polyurethane foams substituted with nano hydroxyapatite and zinc oxide or doped ZnO. Human Embryonic Stem Cell-Mesenchymal progenitors (hES-MPs) and Bone Marrow Stromal cells Y201 were used to evaluate the cell metabolic activity, cell adhesion and cell seeding efficiency.

Results
The ZnO_2 and ZrZnO5 incorporated scaffolds improved the tensile strength from 0.3MPa (PU porous scaffold) to 0.87MPa and 0.81MPa respectively. The cell culture data suggested the ZrZnO5 incorporated scaffolds likely stimulated the cells to proliferate at a faster rate as compared to cells on CeZnO5 and CuZnO5 containing scaffolds. Overall, the doped ZnO incorporated nanoparticles stimulated the Y201 cells to attach and proliferate better on scaffolds as compared to controls. The copper doped ZnO and cerium Doped ZnO incorporated scaffolds were found to be the most efficient when it comes to cell attachment as the highest cell metabolic activity was recorded after 16 hours of cell seeding.

Figure 1: Cell Seeding Efficiency after 16 hours incubation with Y201 cells. A) The metabolic activity assay was performed to identify viable cells attached on scaffolds and Tissue Culture plastic(TCP). B) the percentage cell attachment is shown with control being considered 100%(cells on TCP). CuZnO5 incorporated scaffolds showed 80% cell attachment. Data represents mean±S.D, n= 3, N=3

Conclusions
The ability of doped ZnO nanoparticles to support Y201 cell attachment and proliferation is better than HA alone, combined with previous data indicating their ability to enhance

angiogenesis, indicates their potential for use in improved bone bone tissue engineering scaffolds.

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keywords: zinc oxide, bone regeneration, tissue engineering

62825424546

ELECTROSPUN HYBRID SCAFFOLDS TOWARDS ENHANCED BONE TISSUE REGENERATION

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Introduction
Extracellular matrix (ECM) of hard tissues in human body has a specific composition combining organic and inorganic fractions that determines their properties and function. Therefore, designing scaffolds for bone tissue regeneration we should consider hybrid materials providing proper mechanical and structural support. The focus of current study were scaffolds based on poly(3-hydroxybutyric acid-co-3-hydrovaleric acid) (PHBV) enriched with hydroxyapatite (HA) nanoparticles, prepared by blend electrospinning [1].

Methodology
Two types of electrospun scaffolds were prepared using an EC-DIG device (IME Technologies), namely PHBV and PHBV+HA. For both samples the applied voltage during electrospinning was 17 kV and needle was positioned at distance of 20 cm to the collector. Morphology of as received fibers was studied by scanning electron microscopy (SEM, Meriln, Zeiss) followed by focused ion beam (FIB) sectioning to expose fibers interior. The successful incorporation of HA particles into PHBV fibers was confirmed by energy dispersive X-ray spectroscopy (EDS, Brucker). Finally, in vitro studies with MG-63 cells were performed up to 7 days of incubation. Cell viability and proliferation was evaluated using Cell Titer-Blue® Assay and cell morphology was studied by confocal and SEM imaging.

Results and discussion
Smooth fibers with random orientation and average diameter of 2.92 ± 0.28 μm were obtained from electrospinng of PHBV solution. Addition of HA particles resulted in porous fibers formation with increased diameter of 3.76 ± 0.37 μm. Ceramic particles were present both inside fibers as well as exposed on their surface.

Based on proliferation test and confocal imaging results, the higher cell number was observed on hybrid PHBV+HA scaffolds compared with solely PHBV fibers. Especially significant boost in cell proliferation was observed between 4th and 7th day of incubation. Microscopy imaging enabled to visualize cells elongation, filopodia formation and penetration in depth on both type of scaffolds. Importantly, PHBV+HA fibers due to particles on their surface provided more anchoring points for cells spreading, therefore, higher cell number with longer filopodia were observed for hybrid scaffolds.

Conclusions
In this study we showed that by simple approach of blend electrospinning combining PHBV and HA we could obtain scaffolds improving bone cells proliferation and growth. Therefore, we proved that such composite scaffolds are a very promising material for bone tissue regeneration.

Acknowledgement
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keywords: electrospinning, hybrids, bone regeneration

31412737884

EVALUATION OF THE BIOLOGICAL RESPONSE OF ZRO2 FUNCTIONALIZED MAGNESIUM ALLOYS

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Biodegradable magnesium alloys are promising candidates for temporary fracture fixation devices in orthopedics; nevertheless, its fast degradation rate at the initial stage after implantation remains as one of the main challenges to be resolved. ZrO2-based coatings to reduce the degradation rate of the Mg-implants are an attractive solution since they show high biocompatibility and stability. The thin films of ZrO2 were deposited by magnetron sputtering on a Mg-Zn-Ca alloy was evaluated. The biological response was evaluated through MTT assay, Live Dead assay, and inmersión test. ZrO2 coating does not compromise the biocompatibility of the alloy and permits better cell adhesion and proliferation of mesenchymal stem cells directly on its surface, in comparison to the bare alloy.

keywords: Magnesium Alloys Biocompatibility Zirconium Oxide

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EXTRACELLULAR MATRIX SYNTHESIZED BY DENTAL PULP STEM CELLS – MULTIFUNCTIONAL TOOL FOR BONE REGENERATION

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Introduction
Bone loss due to various infections, aging, tumors or genetic disorders is worldwide problem; moreover, not all defects can be regenerated by using today's technologies. For this reason scientists construct artificial bone scaffolds which could provide new bone treatment strategy. Scaffolds surface biodecoration highly enhances their integration and allows to control damaged tissue regeneration. Hard tissue scaffold coating with cell derived extracellular matrix (ECM) is a rapidly growing field of research. Usually bone scaffolds are decorated by ECMs synthesized by primary bone marrow-derived mesenchymal stem cells (BMSC), adipose stromal cells (ASC), dermal fibroblasts, osteoblast/fibroblast coculture, and osteoblast cell line. It was shown that ECMs formed by cells of osteogenic origin contains additional proteins which even more enhances new bone formation compared to non-bone related MSCs. However, the extraction procedure of BMSCs or osteoblasts is painful and requires additional surgical interventions to the patient's body. One of the possible candidates to overcome this issue could be dental pulp stem cells (DPSCs). So far, there are no studies in which the ECM formed by DPSCs would be detailed analyzed. Therefore, DPSCs-ECM compositions as well as its adaptability for artificial bone construction were studied.

Methodology
Primary rat DPSCs and pulmonary trunk blood vessels endothelial stem cells (PTEC) were used for in vitro studies. DPSCs were seeded on porous polylactic acid (PLA) scaffolds printed by FDM 3D printing technology and grown for 7 days to produce cell secreted ECM. Later, samples were decellularized (PLA+ECM scaffolds) and used for cell-scaffold interaction (adhesion, migration, proliferation) studies, proteomic analysis of ECM composition, and osteo- and angiogenesis induction assessment. Finally, PLA-ECM ability promote new bone formation was evaluated by implanting PLA+ECM scaffolds into the critical-size rat calvaria defects. In all experiments pure PLA scaffolds were used as a control.

Results
Cell-scaffold interaction studies demonstrated that DPSC derived ECM initiates enhanced mesenchymal stem cells (MSC) adhesion, migration, and proliferation. Furthermore, its detail proteomic analysis revealed that proteins retained on PLA scaffolds after decellularization, according to Enrichr database, contributes to biological processes associated with bone/cartilage formation, angiogenesis, ECM formation, immune response, protein processing, and membrane transport. PLA-ECM induced increased ALP activity, osteogenic- and angiogenic-related genes

and proteins expression up-regulation in DPSCs, likewise positive stimulation of PTEC migration confirmed its osteogenic and angiogenic properties in vitro. Finally, in the case of rat calvaria defects DPSCs synthesized ECM recruited endogenous stem cells, thus promoting new bone formation process.

Conclusions
Results showed that PLA-ECM improves surface properties required for initiation of bone self-healing process. Therefore, DPSC secreted ECM can be a promising tool for various scaffolds decoration in the field of bone tissue engineering. Nevertheless, additional studies are necessary were synergistic effects of ECM with different biomaterials formulations would be evaluated.

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keywords: Dental pulp stem cells-derived extracellular matrix, Polylactic acid, Bone regeneration, Proteomic analysis.

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HYBRID ALGINATE-GELATIN SCAFFOLDS WITH ADDITIONAL 3D PRINTED POLYCAPROLACTONE REINFORCEMENT

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Bone is one of the most important composite building materials, which is the main component of the human skeleton [1]. It is estimated that the total number of fractures in the European Union will increase from 2.7 million in 2017 to 3.3 million in 2030. The resulting annual costs associated with fractures are expected to increase by 27%. Furthermore, as the population ages, this figure is expected to increase continuously. In this context, new biomaterials are constantly being developed to regenerate bone fractures and support tissues damaged by progressive osteoporosis. Typically, they are designed to promote optimal integration of bone with the implanted scaffold until complete bone regeneration. Hydrogels are among the most promising biomaterials for tissue engineering applications. In bone tissue engineering, hydrogel materials are used as scaffolds for growth factor transport and cell adhesion [1-3]. This is due to the fact that they show a high ability to mimic natural tissues - higher compared to other biomaterials [4]. It is often difficult to identify a one ideal polymer for the preparation of hydrogels due to the limited possibilities of modifying their properties and for this reason mixes of polymers are increasingly used, which allows modification of their parameters such as mechanical properties or microstructure and surface morphology. Alginate-gelatin binary hydrogels are among the most commonly used materials in tissue engineering [5]. The research work carried out on the influence of alginate-gelatin hydrogel fabrication parameters on the properties of the obtained materials indicated the further need for their modification to improve mechanical properties. One potential solution to this problem is to incorporate additional reinforcing structures into the hydrogel matrix [6], e.g. by introducing additional reinforcement in the form of a 3D printed polycaprolactone (PCL) scaffold. The aim of this study was to develop a hybrid alginate-gelatin scaffold with additional reinforcement in the form of a 3D printed PCL scaffold crosslinked with strontium chloride. The obtained hybrid scaffolds were characterized in terms of mechanical (Young's modulus), biological (in direct and indirect contact with osteoblast-like Saos-2 cells) and physicochemical (degradability) properties. The obtained results confirmed the biocompatibility of the obtained hybrid scaffolds in both indirect and direct test. The introduction of additional reinforcement significantly improved the mechanical properties. The hydrogel material did not separate from the PCL scaffold during degradation testing which indicates a solid bond between these materials. On the basis of the obtained results it was concluded that by introducing an additional scaffold printed with PCL it is possible to obtain hybrid scaffolds of increased stiffness and simultaneously high biocompatibility to support the treatment of bone tissue defects.

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keywords: alginate, gelatin, PCL, hybrid scaffold

31412756007

MAGNESIUM ALLOYS WITH LPSO STRUCTURES AS PROMISING MATERIAL FOR MUSCULOSKELETAL IMPLANTS – CORROSION RESISTANCE EVALUATION

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The growing interest in finding the best way to strengthen Mg alloys has recently focused on the development of Mg-Y-Zn alloys with long-period stacking ordered (LPSO) structures [1]. The microstructure of Mg-Y-Zn containing LPSO structures formed during plastic deformation was found to be beneficial in terms of mechanical properties [2]. The goal of this work is to investigate the role of strengthening phases with novel long period stacking-ordered (LPSO) structures in the corrosion resistance of Mg-Y-Zn alloys. Two Mg-based alloys are investigated: WZ42 containing 3.5 wt.% Y and 1.6 wt.% Zn and WZ104 with a chemical composition of 10 wt.% Y and 3.7 wt.% Zn. Once the predominant corrosion mechanism is known, in further perspective, this would lead to the possibility of a successful application, especially the application that satisfied the targeted function of Mg-Y-Zn alloys mainly for implants regarding diseases of the musculoskeletal system.

To achieve the goal of research, optical microscopy observations of the microstructure of the investigated alloys were performed followed by more detailed scanning electron microscopy (SEM) observations along with energy dispersive spectroscopy (EDX) analysis of the chemical composition. After the prevalent features of the microstructure were determined, their impact on corrosion behavior was investigated. Corrosion tests were performed in a naturally aerated phosphate buffered saline solution (PBS) at 37 ° C. Parameters describing corrosion resistance were obtained using electrochemical testing consisting of open-circuit potentials (OCP), electrochemical impedance spectroscopy (EIS), and the potentiodynamic polarization measurements. Immersion tests with subsequent SEM observations enabled us to describe corrosion mechanisms that occurred on the surface of alloys.

As demonstrated, the dominant morphology of the LPSO phases in WZ42 is block-like, while in WZ104 the thin platelet LPSO phase prevails. Randomly distributed LPSO structures are observed in both alloys. WZ42 contains a smaller volume fraction of LPSO phases than WZ104. The results of the electrochemical tests showed that WZ42 is more corrosion resistant than WZ104. Immersion tests with subsequent SEM observations revealed that the corrosion damage occurred mostly in the close proximity of the LPSO phases. The more LPSO phases formed in the alloy the higher number of areas, where the corrosion is initiated.

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keywords: Mg-Y-Zn alloys, LPSO phases, microstructure, microgalvanic corrosion

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MAGNETIC 3D-BIOPRINTED COMPOSITE SCAFFOLDS BASED ON BIOPOLYMERS, HYDROXYAPATITE AND SPIONS FOR BONE TISSUE REGENERATION

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Introduction. Bone is the second most commonly transplanted tissue. The problems with allografts and xenografts as substitute for original tissue are associated with the graft loosing, inflammation and osteolysis or the death of bone tissue, which only adds to the existing damages [1,2]. In order to solve these shortcomings, tissue engineering have suggested a number of potential therapeutic approaches for restoring bone tissue function by developing biocompatible and long-lasting tissue constructs [3]. In this regard, 3D printing has become of great interest due to its exceptional controllability, reproducibility and unlimited repeatability in fabricating layered scaffolds [4]. The paper presents the obtaining of 3D bioprinted scaffolds based on gelatine methacrylate (GelMa), chitosan methacrylate (CsMa), hyaluronic acid (HA), SPIONs and hydroxyapatite (Hap) and scaffolds evaluation for their applicability in bone regeneration engineering.

Methodology. PBS solutions of GelMa, CsMa and hyaluronic acid were homogenised with photoinitiator, SPIONs and different proportions of hydroxyapatite and bioprinted and crosslinked at 405nm, using a Cellink Bioprinter Inkredible+. Finally, the scaffolds were freeze-dried for characterization. The obtained scaffolds were characterized for their structure (FTIR, XRD), morphology (SEM microscopy), swelling behaviour, mechanical and magnetic properties, in vitro degradability and citocompatibility (osteoblasts).

Results. The scanning electron microscopy indicated a good homogenisation of the components and scaffold with controlled 3D structure and porosity can be obtained by modifying the composition and bioprinting parameters. The scaffolds retain simulated body fluids and are degradable by collagenase.The mechanical properties are strongly dependent by composition and a higher content of Hap increases the mechanical strength.

Conclusions. Composites 3D scaffolds with controlled morphology and biodegradability, with magnetic and mechanical properties have been obtained and tested. The scaffolds are citocompatible and induce cells adhesion and proliferation.

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keywords: 3D bioprinting, composite scaffolds, bone tissue regeneration

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MESENCHYMAL STEM CELL DIFFUSION INTEGRATED MECHANO-BIOLOGY ANALYSIS OF 3D SCAFFOLDS

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Large bone defects remain a clinical challenge to be solved via emerging 3D scaffolds. For effective treatments, the bone scaffold should satisfy competing mechanical, biological, and chemical functions presenting a challenging design problem. Besides mainstream experimental studies, computational efforts exist in literature presenting scaffold designs with primarily optimized geometries focusing on geometry, pore size, and porosity (Dias et al., 2014; Poh et al., 2019) to deliver desired mechanical features in the form of stiffness and permeability. However, one of the most crucial metrics for scaffold design relates to bone regeneration based on mechano-biological behavior (Boccaccio et al., 2016; Metz et al., 2020) which is also experimentally validated. Similarly, in addition to mechano-biological tissue response, incorporation of the MSCs diffusion will provide more realistic approach for determining the most suitable scaffold geometry for effective bone repair (Geris et al., 2004). Therefore, to examine the effect of MSCs diffusion coupled with mechano-biology calculations, here we present a comparative parametric study for a regular structured 3D cubic scaffold structure with aligned square pores. The model is developed via COMSOL Multiphysics® software and a MATLAB interface script. Material properties are updated iteratively until the steady state cell concentration in the scaffold pores are obtained. The effect of diffusion on the bone regeneration is analyzed for scaffold architectures with different pore size values. The wall shear stress, fluid velocity, pressure and octahedral shear strain values were analyzed in detail. Initial results indicate that incorporation of stem cell diffusion leads to altered values of the mechano-biological response demanding optimal pore size values to be determined incorporating these effects for effective bone healing. To the best of our knowledge, this is the first detailed 3D scaffold analysis of coupled mechano-biology and cell diffusion processes and its effect on bone regeneration based on a coupled fluid flow-mechanical analysis of poroelastic scaffolds.

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keywords: bone scaffold, mechano-biology, MSCs diffusion, wall shear stress.

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MULTIFUNCTIONAL COMPOSITE COATINGS SUPPORTING BONE REGENERATION

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So far, most organ and tissue damage resulting from genetic defects or trauma is treated with either pharmacologically or surgically. This is performed through the use of appropriate drugs or, in more severe cases, organ transplantation. However, over the past few years, biomaterials have increasingly gained importance in materials science with great hopes for solving tissue and organ healing problems. These smart bioactive materials, have great potential in regenerative medicine through stimulation of surrounding tissues or local drug delivery. Of particular interest is the coating of implants with specific layers of biomaterial. Such a strategy can be used to increase biocompatibility as well as to provide additional properties without changing the base material. In the case of materials developed for bone regeneration, a coating created from a bioactive hydroxyapatite (HA) ceramic can be used to add the desired osteointegration feature. HA exhibits the ability to bond with natural tissue, which largely eliminates the danger of implant loosening. Moreover, it is highly biocompatible and thus does not cause any allergic reactions. Unfortunately, HA itself has low mechanical strength and high fragility, which limits its application. A solution to this problem may be to suspend HA in a polymer matrix that provides flexibility. The polymer phase is able to transfer the stresses and the resulting composite will not lose its bioactive properties due to the nature of HA. Furthermore, the nature of the polymeric phase is interesting, because due to the structure and arrangement of the polymeric chain, it can be modified with additional active ingredients such as proteins or drugs, and thus, it is possible to use such a biomaterial as a carrier of an active substance.
In this study, innovative ceramic-polymer composite coatings based on HA, polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) containing glutathione and collagen were developed for bone tissue regeneration. Glutathione is a tripeptide that exhibits antioxidant and health-promoting properties as well as supports osteoblast differentiation, while collagen is the main component of the intercellular substance of organisms and creates the organic phase of natural bone. Bioactive HA was suspended in the polymeric phase and the multicomponent material was then applied to polylactide (PLA) plates. This way, a multifunctional material was obtained showing potential abilities to support the process of implant connection with host tissues as well as to support regeneration of damaged tissues.
Physicochemical analysis including incubation studies, FT-IR or SEM was performed. In addition, tribological properties and cytotoxicity were investigated. The materials have been found to be safe in contact with l929 mouse fibroblast cells. Moreover, as a result of incubation in SBF fluid, the appearance of new apatite layers on the surface was observed which confirms the occurring biomineralization process.
Considering the promising results obtained, the material can be subjected to further research.

keywords: Coatings, hydroxyapatite, collagen, bone

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PHYSICOCHEMICAL AND BIOLOGICAL ANALYSIS OF SYNTHETIC HYDROXYAPATITE OBTAINED VIA A WET PRECIPITATION TECHNIQUE

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Along with the continuous development of civilization and the intensive technological progress, the diseases of civilization are becoming a growing problem. The most common diseases of civilization include cardiovascular diseases, cancer and osteoporosis [1]. According to the International Osteoporosis Foundation, the problem of osteoporosis affects approx. 200 million people. Osteoporosis is a disease characterized by the loss of bone density. As a result, the weakened bone fractures due to very small injuries. In many cases these fractures lead to the need for a suitable prosthesis [2]. An important material very often used in regenerative medicine and implantology is hydroxyapatite (HAp). It is a material which, by its chemical composition, corresponds to an inorganic part of bone. It is called a bone replacement material and is used to make coatings for metal implants. Moreover, HAp is characterized by osteoconductivity, i.e. the ability to support the formation of new bone tissue [3]. In recent years, multifunctional implants have become increasingly popular, which in addition to filling the bone defect are designed to provide the bone with appropriate active substances. Due to its properties such as biocompatibility, non-toxicity and the possibility of set with bone tissue, hydroxyapatite is an interesting material to use as a component of multifunctional implants [4].

In this work, hydroxyapatite was obtained using a wet precipitation method. Ammonium dihydrogen phosphate and hydrated calcium nitrate were used as HAp precursors. The syntheses were performed for different conditions, changing the pH of the reaction mixture and the concentration of the reagents applied, respectively. After selecting the most appropriate reaction conditions, obtained hydroxyapatite powder was compared to the commercial powder while the particular attention was paid to such characteristics as the phase purity, the surface development, morphology, and particle size distribution.

As a result of performed investigations it was concluded that synthetic hydroxyapatite was characterized by 71.8% more specific surface area than commercial powder. Furthermore, it was proved that the size of the particles of obtained HAp was within the range of 1 - 100 μm while in the case of commercial powder two types of particles were observed, i.e. with a size ranging from 1 – μm and 150 – 600 μm.

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keywords: biomaterials, hydroxyapatite, ceramic

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RECREATING BONE EXTRACELLULAR MATRIX WITH PEG HYDROGELS FUNCTIONALIZED WITH BIOMIMETIC MULTIFUNCTIONAL PEPTIDES

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Introduction
Synthetic hydrogels are a promising strategy to reproduce the 3D microenvironment of bone tissue. However, most of them lack the required bioactivity to actively influence cell fate. To this end, biochemical cues, such as integrin-binding molecules combined with growth factor derived sequences, may be installed on hydrogels to mimic bone extracellular matrix (ECM) [1-2], thus improving their bioactivity. Herein, we engineered a PEG-based hydrogel modified with a biomimetic peptide containing the cyclic RGD cell adhesive motif (cRGD) and a BMP-2 derived peptide (DWIVA or cDWIVA) in a well-defined chemical manner. The hydrogels also incorporated matrix metalloproteinases (MMPs)-degradable sequences to allow their biodegradation and to ensure cell spreading and differentiation. In vitro studies demonstrated the capacity of such biomimetic hydrogel to support cell survival, growth and spreading and, more interestingly, to trigger human mesenchymal stem cells (hMSCs) osteogenic differentiation.

Methodology
The thiolated biomimetic peptides (cRGD-DWIVA and cRGD-cDWIVA) were synthetized by solid-phase peptide synthesis and anchored to 4-arm-PEG molecules with maleimide groups via Michael addition. Hydrogel crosslinking was also achieved via Michael addition by mixing thiolated-PEG/-MMPs-degradable sequences with functionalized PEG molecules. Physicochemical properties of hydrogels were assessed by means of rheological measurements, scanning electron microscopy as well as degradation and swelling assays, while their biological performance was evaluated with viability, immunostaining, ALP activity and gene expression studies with hMSCs.

Results
Preliminary studies were conducted to find the optimal ratio of biomimetic peptide, biodegradable crosslinking and percentage of PEG in order to engineer advanced hydrogels for bone tissue engineering. After establishing the optimal hydrogel compositions, their physicochemical characterization confirmed the presence of the biomimetic peptides as well as the desired mechanical properties and degradation rates to support cell proliferation and osteogenic differentiation. In this regard, control hydrogels without biomimetic peptides nor biodegradable crosslinking supported cell survival but failed to promote cell spreading or differentiation. On the contrary, the biomimetic-degradable hydrogels promoted high values of

hMSCs proliferation and spreading. More interestingly, the functionalized hydrogels triggered hMSCs osteogenic differentiation, as demonstrated by a significant increase in ALP activity and an overexpression of osteospecific genes.

Conclusions
The functionalization of PEG hydrogels with biomimetic peptides combining the cRGD sequence with BMP-2-derived motifs resulted in novel biomaterials with the required mechanical properties, biodegradability rates as well as the biochemical cues to recreate bone ECM and support hMSCs osteogenic differentiation. Thus, this hydrogel may be a promising alternative to the current therapies with stem cells in the field of bone regeneration.

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keywords: PEG hydrogel, RGD, DWIVA, peptides, osteogenic differentiation

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SCAFFOLDS BASED ON TRICALCIUM PHOSPHATE AND BACTERIA-DERIVED POLYHYDROXYOCTANOATE – CYTOCOMPATIBILITY STUDIES

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Tissue engineering faces a major challenge in providing novel, functional materials that will meet the requirements of tissue engineering. These properties can be provided by tricalcium phosphate-based bioceramics (TCP). The combination of a ceramic matrix with polymers creates great opportunities to improve the physicochemical and biological properties of the obtained composites. Polyhydroxyoctanoate (PHO) belongs to the polyhydroxyalkanoates (PHAs) family. Due to its degradation products – nourishing (R)-3-hydroxyacids[1], the application of this novel elastomeric polymer opens new routes for promoting tissue regeneration.
In this study, the bioceramic/polymer based scaffolds (TCP/PHO) were prepared as previously described[2]. To assess the possibility of using TCP/PHO composites as bone tissue substitutes, their biocompatibility was tested using MC3T3-E1 mouse preosteoblastic cell line. Cells were cultured in Minimal Essential Medium Eagle medium with alpha modification - α-MEM supplemented with 10% fetal bovine serum - FBS and antibiotics: penicillin/streptomycin at 37°C at 5% CO2 concentration in an incubator. Materials' samples were incubated in α-MEM for 24 hours at 37°C in 5% CO2. MC3T3-E1 cells were seeded in a 96-well plate at 25·103 cells per well and incubated in 150 μl α-MEM for 24 hours. Then, the media was exchanged with extracts obtained by incubating material samples in α-MEM. Cells were cultured for 24 hours. The negative control was cells cultured in medium alone (MEM). The cell viability assay (Alamar Blue) was performed.
In the case of supernatants from TCP scaffolds, cell viability was 117.11 ± 5.59%. which is significantly higher in comparison to the negative control (100% viability was determined for cells cultured in α-MEM medium alone). This result indicates that the extract of TCP material has a beneficial effect on cells during the first 24 hours. The TCP/PHO scaffolds were fully biocompatible and cell viability was comparable to that of cells in α-MEM. Cell survival in extracts of polymer-infiltrated ceramic (TCP/PHO) was 105.15 ± 3.62%. The reason for these differences is the hydrophobic nature of polyhydroxyoctanoate.
The obtained results suggest that both bioceramic and composite scaffolds are fully cytocompatible.

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keywords: tricalcium phosphate, polyhydroxyalkanoates, scaffolds, cytocompatibility

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SELECTION OF SUITABLE CONDITIONS FOR STABILIZATION OF POROUS CHITOSAN STRUCTURES WITH THE USE OF VANILLIN FOR REGENERATIVE MEDICINE APPLICATIONS

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Chitosan is a well-known polymer widely used in tissue engineering and regenerative medicine. It is biocompatible, biodegradable, non-toxic, has antibacterial and osteoconductive properties. It is perfect for the production of implantable materials, including porous scaffolds. Unfortunately, as with most natural polymers, chitosan structures exhibit poor mechanical properties, low durability, and rapid degradation. To counteract these problems, chitosan is sometimes combined with fillers, e.g. bioglass particles, to form more durable composites. However, the best method of obtaining stable chitosan structures is to carry out cross-linking processes. Methods of cross-linking chitosan, for example by creating chemical bonds or physical cross-linking, directly between the polymer chains or between the polymer chains and molecules of multifunctional cross-linkers, are known [1]. There are also methods of stabilizing ready-made chitosan scaffolds. The cross-linking density and the properties of the obtained structures depend on the cross-linking method, the type and amount of the cross-linking agent used, as well as the process conditions. The absence of a cytotoxic effect is also an important aspect in the selection of a cross-linking agent for biomaterials.
The natural crosslinking agent for chitosan can be 4-hydroxy-3-methoxybenzaldehyde known as vanillin (VAN). The chemical cross-linking reaction of chitosan with vanillin is based on a reaction involving the amine groups of chitosan and the aldehyde group of vanillin, resulting in the formation of a Schiff's base and a color change from light yellow to strong yellow. Besides, the vanillin hydroxyl group can also form a hydrogen bond with the hydroxyl or amino group of chitosan [2]. As a result of these reactions, a dense three-dimensional polymer network is formed, which stabilizes the chitosan structures.
The aim of the study was to determine the optimal conditions for stabilization/cross-linking of chitosan/bioglass composites with the use of a natural cross-linking agent - vanillin, in terms of obtaining mechanically stable three-dimensional porous scaffolds with porosity suitable for the colonization and proliferation of cells and showing no cytotoxic effect.
As part of the work, cross-linking of chitosan with the use of vanillin as a component of composite masses as well as the stabilization of porous chitosan structures with the use of

vanillin solutions was carried out. Cross-linked and/or vanillin-stabilized composites were tested for microstructure (SEM imaging and mean pore size determination), water absorption and solubility tests, mechanical compressive strength tests, and cytotoxicity and proliferation tests. It has been shown that properly selected conditions of the cross-linking and/or stabilization process with the participation of vanillin allow to obtain durable and mechanically resistant chitosan scaffolds with an appropriate microstructure and desired biological properties.

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keywords: chitosan, vanillin, crosslinking, stabilization

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NANOPARTICLE SIZE EFFECT IN THE PROPERTIES OF NANO-BIOMATERIALS FOR BONE TISSUE REGENERATION

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INTRODUCTION
Millions of bone reconstruction operations are conducted every year. A promising alternative to autologous bone grafts are customized bone scaffolds. Biofunctional custom implants fills the defect in the tissue and carry mechanical loads. The presence of groups with hydrophilic properties (e.g., -OH) on biomaterial surface is preferred. For this reason, the modification of the surface of polymeric and metal materials is becoming more and more popular. In the field of regenerative medicine, homogeneous, biocompatible, bioactive coatings stimulating the regeneration of bone or cartilage tissue. All these conditions are met by nano-hydroxyapatite layers deposited by sonocoating method. Hydroxyapatite (HAP) is the main mineral component of the bone, responsible for the stiffness and mechanical strength.

METHODS
GoHAP NPs were obtained using the hydrothermal microwave synthesis method described in detail by Kusnieruk et al. [1]. The fabricated nanoparticles were used in the process of coating the surfaces of titanium and polymeric implants by sonochemical method. The method of sonochemical deposition of hydroxyapatite layers was described by B. Wozniak, U. Szalaj, et al. [2] Three types of obtained nanoparticles, differing in particle size, were separately selected for coating: GoHAP with the particle size about 10 nm, 15 nm, 45 nm. As a cotrol commercial HA Biocer was used.

RESULTS
This work concerns a unique eco-friendly microwave synthesis process enabling strict size control of hydroxyapatite nanoparticles (GoHAP™) in the range of 10±1 to 42±4 nm by controlling the synthesis parameters such as time, pressure and temperature. The complete characterization of GoHAP™ nanoparticles and the relationship between material properties and particle size has been demonstrated. This work presents the mechanism of formation of the nanoparticles layer deposited by sonocoating, as well as the relationship between the size of nanoparticles used in the coating process and the properties of the deposited layer. The presentation shows the kinetics of the nanoparticles layer deposition process depending on the GoHAP nanoparticles size as well as the properties of the obtained layers, such as morphology or contact angle, biocompatibility.

DISCUSSION & CONCLUSIONS
In case of hydroxyapatite nanoparticles, the particle size affects the efficiency of the sonocoating deposition process as well as the properties of the hydroxyapatite layers. The use of size-controlled nanoparticles makes it possible to control the properties of the biomaterial, e.g. the amount of calcium ions released by using nanoparticles of different sizes and dissolution times.

For this reason, special attention should be paid to the nanoparticle size in the design of new materials for bone tissue regeneration.

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BIOINSPIRED NERVE GUIDANCE CONDUITS FOR OPTIMAL NERVE REGENERATION USING POLYHYDROXYALKANOATES

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Introduction
Peripheral nerve injuries (PNIs) less than 5 mm in length self-repair compared with those with wider gaps1,2. Traditionally, PNIs of 20 mm or less have been repaired either by suturing nerves end-to-end, or by grafting. However, these techniques present surgical and patient recovery drawbacks, prompting the search for better technologies. Nerve guidance conduits (NGCs) have shown promise to mitigate these limitations but are still deficient in supporting satisfactory recovery of PNIs due to poor fit between the NGCs and the local environment. This project aims to address this by developing a biomimetic nerve guidance prototype with improved physical and biochemical support for optimal PNI recovery using highly biocompatible and bioresorbable Polyhydroxyalkanoates (PHAs).

Methodology
The PHAs, mcl-PHA and P(3HB) were produced via bacterial fermentation in a 30L Solaris bioreactor and characterised using techniques such as FTIR, NMR, DSC, Tensile testing etc. Two-dimensional films were produced by solvent casting method and assessed for their biocompatibility using NG108-15 cells. Nerve guidance conduits were produced by two methods including fused deposition modelling based 3D printing using the Cellink BioX printer and dip moulding using an automatic controlled dip moulding machine.

Results
Production of the mcl-PHA resulted in a high titre and productivity. This productivity was about 15-fold higher in comparison with the reported values in literature. Mechanical analysis showed that the mcl-PHA had a Young's modulus of 10 MPa and a maximum elongation at break of 630% compared to 588.8 MPa and 33.2% of P(3HB). The polymers have been 3D printed into NGCs with high fidelity. Similarly, a blend of the mcl-PHA and P(3HB) was successfully dip moulded into uniform tubes that were initially tested in vivo and found to be very suturable. The biological characterization of the flat films of PHAs demonstrated that the polymers have excellent biocompatibility with NG108-15 cells. Neat films of the mcl-PHA resulted in higher cell viability than tissue culture plastic by day 6. Additionally, confocal imaging of immunolabelled samples revealed that the PHAs highly supported neural extension.

Conclusion
Higher productivity and yields were achieved for the production of PHAs compared to values reported in literature. The polymers produced were highly biocompatible with NG108-15 neuronal cells and supported longer neurite extension in comparison with the tissue culture plastic. Also, the polymers were successfully 3D printed and dip moulded into uniform NGCs with desired dimensions. Initial in vivo studies showed that the polymer was very suturable and suitable for further studies into their potential use in the production of a next generation nerve guidance conduit.

keywords: PNI, NGC, PHAs, 3D printing, in vivo

73296344805

CARBON NON-WOVEN SCAFFOLDS COATED WITH AN IRON NANOLAYER USED IN MONITORING OF THE CARTILAGE REGENERATION PROCESS

Roksana Kurpanik (AGH University of Science and Technology, Krakowe, Poland), Weronika Mazur (AGH University of Science and Technology, Krakow, Poland), Artur Krzyżak (AGH University of Science and Technology, Krakow, Poland), Anna Ścisłowska-Czarnecka (Academy of Physical Education, Krakow, Poland), Marcin Kudzin (Łukasiewicz Research Network – Textile Research Institute, Łodz, Poland), Maciej Boguń (Łukasiewicz Research Network – Textile Research Institute, Łodz, Poland), Ewa Stodolak-Zych (AGH University of Science and Technology, Krakow, Poland)

Introduction
Regeneration of cartilage tissue is a difficult and lengthy process. An active fibrous substrate that mimics the random arrangement of fibres in the extracellular matrix (ECM) may be a promising strategy to aid the self-repair process. Fibrils form interconnected, three-dimensional network whose morphology promotes oxygen and nutrient delivery to cells. Tuning the surface topology and chemistry of the scaffolds affects cell functions such as attachment, proliferation etc.
Aim of our work was to obtain a nonwoven carbon fabric modified with iron layer to monitor the process of regeneration of cartilage or bone defects by MRI imaging.

Methodology
A two-step thermal conversion process was used for the thermal treatment of polyacrylonitrile nonwoven substrate (PAN, Sigma-Aldrich). The first step was oxidation, the second step was low-temperature carbonization. The samples were modified using the DC magnetron sputtering system with metallic layers deposited in different time. The presence of the layer and its uniform distribution on the fibre surface were verified by SEM/EDS and MRI. T1 and T2 relaxation times were measured on Rock Core Analyzer (Magritek), they were obtained from distributions calculated by applying Inverse Laplace Transform. Effect of the layer on physicochemical properties; specific surface area, contact angle, surface free energy and porosity was evaluated. Biocompatibility test was performed by contacting cells with the scaffold after 3, 7 days using osteoblast and chondrocytes.

Results
The thermal conversion of the polymer precursor results in 6% fibres shrinkage. The carbon fibres show a significant decrease in wettability compared to the polymer nonwoven. Magnetron sputtering leads to the formation of a metallic layer on the side of the nonwoven exposed to the source, which is heterogeneous in nature: mesopores are present on the surface. Their presence has a positive effect on cells viability and proliferation. Along with the evaporation time there is a smoothing of the fibres surface with a continuous layer. The longer the deposition time, the better cell morphology, which adheres to the fibres surface and spreads between them. Their wettability changes and is near 80o. The layer has a positive effect on texture of the material and physicochemical properties improving cellular response

Conclusion(s)
The carbon non-woven substrates formed by thermal conversion of polymer precursor can be modified by magnetron sputtering. The metallic layer influences texture of the carbon fibres; the shorter the sputtering time the rougher the fibres surface increases improving the cellular response from osteoblasts and chondrocytes. The layer is external with thickness depending on the process conditions. The iron layer increases the effectiveness of MRI imaging.

Acknowledgments
The work was supported by the National Science Centre Poland (NCN): Project no UMO-2018/31/B/ST8/02418

keywords: carbon fibers, MRI, cartilage defect, bone defect, chondrocyte, osteoblast, tissue regeneration

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keywords: carbon fibers, MRI, cartilage defect, bone defect, chondrocyte, osteoblast, tissue regeneration

62825438488

CELL PERFORMANCE ON GRADIENT MELT-ELECTROWRITTEN SCAFFOLDS

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Introduction
Human tissues are structurally and compositionally heterogeneous, often consisting of gradients in architecture, extracellular matrix constituents, cell phenotypes, and biochemical factors. Mimicking the structural complexity through scaffold designs for in-vitro tissue regeneration is challenging and yet to be achieved. Melt Electrowriting (MEW) is a high-resolution additive manufacturing method that holds great promise for reconstructing such native gradients. It combines 3D printing and electrospinning principles, allowing thermoplastic polymers to print with unrepresented precision.

The project aims to obtain the gradient scaffolds with different designs using the MEW technique and define their detailed mechanical and biological properties.

Methodology
MEW was used to fabricate micro-porous polycaprolactone (PCL) scaffolds with different pore sizes and fiber orientations with similar dimensions of tendons/ligaments collagen fibers (10 - 20µm in diameter). Light microscopy, scanning electron microscopy, and tensile tests were performed to investigate the morphological and mechanical properties. Most importantly, a comprehensive biological evaluation was done by culturing mouse fibroblasts (NIH3T3) on MEW scaffolds: life/dead assay, Alamar Blue assay, and immunostaining were performed.

Results
Scaffolds with different fiber orientations and pore sizes were obtained with high precision. Uniaxial tensile tests revealed different elasticities of the scaffolds depending on the printed design. The cell studies demonstrated that the MEW PCL substrates are biocompatible. Cell attachment to fibers and pore bridging were improved for the scaffolds coated with Poly-D-Lysine. Cell performance was dependent on the design proposed.

Conclusion
The mechanical and biological performance of the MEW printed scaffolds is dependent on the proposed design and can be tuned in a tissue-specific manner. In future studies, cell attachment and pore bridging kinetics between fibers will be optimized further by adding functional groups on the surface of MEW PCL scaffolds and using different materials for printing. By printing anatomically relevant architectures closer mimics to native tissues can be obtained.

keywords: Melt Electrowriting, complex tissue engineering, Biofabrication, Polycaprolactone

62825425284

FUNCTIONALIZATION OF SURGICAL MESHES WITH BIOENGINEERED SPIDER SILK PROTEINS TO IMPAIR SURGICAL SITE INFECTIONS

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Introduction: The reconstruction of the abdominal wall after abdominal surgery [NN1] results in the loss of tissue biomechanical properties and the occurrence of surgical site infections (SSI). Current available solutions (i.e. implantation of surgical meshes) can restore the abdominal tissue wall biomechanics although inducing significant clinical complications. Bioengineered spider silk proteins offer great potential as drug-free biomaterials [1]. The fusion with human-derived antimicrobial peptides (AMP) represents an innovative approach to render them with antimicrobial activity [1]. Herein, we explore the antimicrobial potential of immobilized bioengineered spider silk proteins with AMP on nanofibrous meshes (NFM) aiming to simultaneously prevent infections and enable to restore the abdominal wall biomechanics.

Methodology: A facile functionalization method comprising the immobilization of bioengineered spider silk protein with antimicrobial peptide (6mer-HNP1), as well as bioengineered spider silk protein alone (6mer), on electrospun polycaprolactone (PCL) NFM was used[2]. The surface of PCL NFM was activated by exposing both sides to ultraviolet ozone for 2 min each. The production of the bioengineered proteins 6mer and 6mer-HNP1 were induced and purified by an immobilized metal affinity chromatography (IMAC)[1]. It was determined the protein immobilization capacity over the activated NFM and the functionalized mesh was further characterized in terms of antibacterial activity and cytocompatibility [2].

Results: The maximum immobilization capacity of the bioengineered proteins 6mer and 6mer-HNP1 were 200 μg mL–1 and 250 μg mL–1, respectively. The immobilization of the proteins on the NFM showed no effect on the formation of beta-sheets by the spider silk domain. Functionalized meshes with 6mer-HNP1 inhibited significantly the adherence and formation of biofilm of Methicillin-Resistant Staphylococcus aureus (MRSA) and Escherichia coli (E. coli), demonstrating their anti-infective potential. The cytocompatibility of the functionalized meshes was validated in vitro in cell studies using a human umbilical vein cell line (EA.hy926) and supported the proliferation of muscle-related cells (Human aortic smooth muscle cells).

Conclusion(s): NFM functionalized with the 6mer-HNP1 showed a significant inhibitory effect against MRSA and E. coli, evidencing their anti-infective properties. The growth and proliferation of human endothelial and muscle cells further evidence their potential for biomedical applications. Overall, this study demonstrates that the use of functionalized meshes with bioengineered spider silk proteins can be a safe and effective alternative to the development of high-performance surgical meshes for challenging abdominal wall repair surgeries.

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keywords: Antibacterial properties, Electrospun nanofibrous mesh, Spider silk, Surface biofunctionalization, Surgery repair

52354542155

GRAPHENE OXIDE TUNES THE RHEOLOGICAL PROPERTIES OF ECM-DERIVED HYDROGELS

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Hydrogels derived from extracellular matrices (ECM-gels) have been described with promising features to promote tissue regeneration, since they preserve several components of native tissues. However, their weak mechanical properties impair ECM-gels use in load-bearing applications. Previously, we have shown that the incorporation of oxidized forms of graphene, one of the strongest materials in the world, in decellularized arteries, improves their mechanical and biological properties [1]. Herein, we propose the incorporation of graphene-based materials (GBMs) in ECM-gels to surpass their weak mechanical properties, allowing their future use as self-standing scaffolds for different load-bearing tissue engineering applications.
GBMs with different lateral sizes were either purchased or produced by modified Hummers' method and characterized by TEM, XPS, XRD, and DLS [2]. Human placenta chorion was isolated and decellularized by a freeze-thawing cycle, followed by an osmotic shock and treatments with triton and DNAse. Materials were sterilized and ECM pre-gels were obtained by digesting the decellularized tissues with pepsin. Decellularization efficiency was evaluated by quantified the DNA amount. Different amounts of GBMs (1-4% v/v) powders (freeze-dried) or aqueous dispersions were incorporated in ECM and mixed(ECM/GBMs pre-gels). Pre-gels were put into a mold and left overnight at 37ºC to promote gelation. Surface topography of ECM/GBMs-gels was evaluated by SEM, and rheological properties were evaluated using single frequency strain-controlled tests, with a 1% strain, 1 Hz frequency, and 5 min testing time. Adhesion of Staphylococcus aureus was assessed by SEM and the coagulation time after contact with recalcified human plasma evaluated.

After decellularization ECM-Gels exhibit 2.2% of remaining DNA comparing with original tissue. Oxidized forms of GBMs dispersed better on ECM gels than non-oxidized. Thus, graphene oxide (GO)was chosen to perform the following characterization steps. SEM images showed that the GO can intercalate ECM-gels collagen fibers. Regarding the rheological properties, a higher increase in complex modulus was observed when GO was incorporated in ECM as a dispersion compared with as a powder.This could be explained by the inability of GO to re-disperse in ECM upon the freeze-drying step. For both conditions(powders/dispersion), ECM complex modulus increased exponentially within the amount of GO. For the highest tested concentration of

GO dispersion(4% v/v), a remarkable increase of 21768% in complex modulus was observed. Even though GO has been described as antimicrobial and/or being able to decrease bacterial adhesion, our results showed that the number of adherent bacteria is similar in ECM and ECM/GO.This may be explained because GO is not exposed on the surface, as observed for PU/GBMs [3].Incorporation of GO in ECM decreased the clotting time of recalcified Human plasma, suggesting that the ECM-gels are more pro-coagulant.
Overall, GO has an effective role in improving ECM mechanical properties, which is a step forward to enable ECM gels in load-bearing applications.

Acknowledgments:
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keywords: Graphene, Mechanical properties, Extracellular matrixes, Tissue Engineering

94238101955

IMPROVED ALGINATE BIOINK BY ENRICHMENT WITH RECOMBINANT SPIDER SILK

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Alginate, a natural linear polysaccharide polymer, extracted from brown seaweed, is extensively applied due to its biocompatibility, all- aqueous ease of handling and relatively low costs. Alginate easily forms a hydrogel when crosslinked with a bivalent ion, as calcium. However, Alginate hydrogel holds low mechanical properties and it is cell-inert. To overcome these drawbacks and to improve alginate as a bioink for bioprinting, we produced a new alginate matrix combined with spider silk, one of the most resilient, elastic, strong materials known to men. Recombinant spider silk biopolymer has a sponge-like structure and is known to be biocompatible and non-immunogenic.

Our results indicated that combining synthetic spider-silk, into bio-printed cell-seeded alginate hydrogels resulted in improved properties compared to alginate: improved mechanical properties of the matrix, achieving a tunable gel viscosity and high printability, alongside prolonged and higher cell viability in culture, probably due to the improved cell-matrix interactions. The new bioink was then used for a bilayer bioprinting of epithelial and stromal endometrial cells. Such a co-culture model will be used for the formation of the complex endometrial tissue, for studying embryo implantation process.

keywords: Alginate, bioink, bioprinting, Spider-silk, cell culture

52354520364

VALORIZATION OF LEVULINIC ACID PLATFORM THROUGH ELECTROSPINNING FIBROUS MEMBRANES FOR IN VITRO MODELLING OF BIOLOGICAL BARRIERS

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In vitro models of biological barriers (e.g., lung epithelium, intestinal epithelium) provide an important benchmark for studying the physiopathological processes (e.g., nutrient and metabolite exchange, interactions with external virus or bacteria) involved in the development of several diseases (e.g., respiratory tract infections, carcinomas). These models represent a reliable platform for a more rapid identification of the most customizable pharmaceutical therapies for their treatment.

Producing sustainable in vitro models obtained from solvents and biopolymers derived from industrial by-products add an important value to this underestimated source of valuable (bio) materials. This works aims at demonstrating the suitability of processing together solvents derived from levulinic acid (LA) (extracted from biomasses) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (synthesized by bacteria strains and whose production is facilitated by LA) to produce electrospun membranes as proof-of-concept of sustainable, engineered biological barrier fully derived from LA as starting feedstock.

Preliminary experiments were performed to identify the most suitable LA-derived solvents (ɤ-valerolactone, 2-methyltetrahydrofuran, methyl ethyl ketone, methyl and ethyl levulinate) and PHBV concentration for obtaining homogenous solutions processable by electrospinning. To enhance the solubility of PHBV in the LA-derivative solvents, formic acid (FA) (co-product of LA industrial synthesis) was added at various volume ratios obtaining binary solvents. Among the tested solutions, PHBV (200 mg mL-1) in MEK/FA (volume ratio 50:50) was the most suitable for the goal of this work.

The electrospinning process was further improved by identifying the optimal process parameters (e.g., applied voltage, spinneret-collector distance, flow rate), and a customized heating system to maintain the PHBV solution in MEK/FA in liquid phase (at 60 °C) was designed and developed on purpose. Self-supporting and microporous mats with micropore size comprises between 1-7 µm were successfully electrospun. These mats show irregular and flat fibers that are partially fused due to not complete evaporation of MEK/FA solvent. Moreover, these mats show an average elastic modulus of 75 MPa and an hydrophobic contact angle of 115° comparable to other electrospun PHBV mats reported in literature [1,2]. Cell experiments demonstrated that the developed fibrous PHBV mats do not negatively alter cell viability of

A549 adenocarcinomic human alveolar basal epithelial cells which adhere and proliferate on the surface of the PHBV mats forming a confluent monolayer of epithelial cells after 48 h.

Globally, these results show for the first time the great potential of converting sustainable solvents and biopolymers, both derived from LA as starting feedstock, into added-value microporous membranes which can potentially be used as sustainable in vitro models of biological barriers.

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keywords: levulinic acid, PHBV, valorization, electrospinning, biological barrier, green solvents

PS46
New developments of regenerative and tissue modeling products

31412706966

A NOVEL NEEDLE−FREE TECHNOLOGY WATERJET BY IMPROVED DELIVERY TO TRANSPORT MUSCLE−DERIVED CELLS TO THE URETHRAL SPHINCTER OF LIVING PIGS

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Stress urinary incontinence is the most common type of urinary incontinence. Actual standard therapeutic modalitie offer symptomatic relief without treating the underlying disorder. Therefore, we developed a novel technology to apply cells for recovery of the urethral sphincter by waterjet (WJ). Based on our previous study in cadaveric samples1, we investigated if a) porcine muscle−derived cells (pMDC) could be injected by WJ in living pig urethrae with high viability, b) the WJ inherits the risk of full tissue penetration of the urethra and thus loss of cells, and c) WJ grants improved precision of cell injection and distribution in tissues targeted.

The pMDC were produced from boars as described1, labeled by PKH26, injected in living female urethrae by WJ (Erbe) using either a moderate (E60−10; n = 18) or elevated pressure (E80−10; n = 6) protocol, and follow-up (f/u) of up to 7 days. Cell injections targeted the site of the maximum urethral closure pressure (Aquarius TT, Laborie Medical). After harvesting the whole bladder and urethra, cells were traced by In Vivo Imaging System (IVIS) and visualized by fluorescence microscopy of cryosections. Nuclei were stained by DAPI, muscular tissue by phalloidin-iFluor 488. The pSRY gene was detected by PCR. The distribution of injected pMDC was measured as X−depth, Y−width, Z−height and calculated areas in the XY-, YZ-, XZ- planes were analyzed. The injection depth of cells in the urethra was measured simultaneously.

We report that the success rates of WJ cell application in living animals were significantly higher (≥ 95%, n = 24) when compared to needle injections2. Only one out of six samples with full penetration was observed in the WJ E80−10group. pMDC kept intact appearing nuclei as stained by DAPI and cellular bodies co−localized as determined by phalloidin-iFluor 488 and PHK26 staining. Intact Y-chromosomes were confirmed by pSRY PCR. The analyses of the 3D distribution of cells after WJ injection documented that the Y−width of the WJ E80-10 group was statistically significant wider ($P = 0.0479$) than that of WJ E60-10 group. The same was recorded for cell distribution in Z-height ($P < 0.0001$). The YZ-plane of the WJ E80−10 group was statistically significant larger ($P = 0.0005$) than that of WJ E60-10group, as well as XZ-plane ($P = 0.0204$). The injection depth of WJ E80-10 compared to WJ E60-10 showed a statistically significant decrease in length ($P < 0.0001$). This indicated that WJ E80-10 injections transported cells closer to the targeted rhabdosphincter, but at a higher risk for full penetration.

We conclude that the novel WJ is a fast, precise, and easy-to-use innovative method to inject living cells in tissues with a significantly wider and diffuse distribution, with less disintegration of the tissue targeted, and at higher success rates. Future studies will investigate the feasibility of the WJ technology in a model of stress urinary incontinence3.

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keywords: myoblast injection, waterjet technology, cell therapy, urinary incontinence, porcine model

94238109768

DEVELOPMENT OF AN 3D BIOPRINTED SKIN MODEL ALTERNATIVE WTIHOUT THE USE OF ANIMAL-DERIVED COMPONENTS

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Introduction
As a result of animal welfare activism and public pressure, policy makers stablished a cosmetic ban for testing and marketing of finished cosmetic products and cosmetic ingredients tested on animals. This motivated the advancement of the scientific progress in this area and with it the creation of alternative models, specially of skin tissue since they are used in regulatory testing as well as in regenerative medicine applications. These skin models are a replacement to the use of animal models. Despite their great impact, there is an increasing need for models that allow the study of more complex physiological and pathological conditions. In this regard, 3D bioprinting offers the opportunity to create structures that resemble the histology of native tissues by allowing controlled spatial cell deposition, in combination of the use of relevant biomaterials, therefore, it is an important technique to achieve complexity in tissue models.
In general, some previously reported 3D bioprinted skin models and alternative skin models still contain “hidden” animal-derived components. In this study, we have focused on creating a 3D bioprinted skin model avoiding the use of animal-derived components. The skin model was created using a cellulose based bioink and the validation of the model was performed using animal-free reagents. The 3D bioprinted skin model was studied to determine its resemblance to native skin tissue.

Methodology
Human dermal fibroblasts (HDFs) and normal human epidermal keratinocytes (NHEK) were expanded under 2D conditions prior to bioprinting. 3D bioprinting was performed using a BIO X bioprinter, HDFs formed the dermal layer and NHEKs the epidermal layer. The 3D bioprinted skin constructs were cultured for 14 days, of which the last 7 days, were in an air-liquid interface. The samples were analyzed on day 14. Cell health in the 3D construct was determined using live/dead assay. Analysis to assess the resemblance of the 3D model to native skin was performed using epidermal cell markers.

Results
The 3D bioprinted constructs presented high cell viability, suggesting the bioink and bioprinting process did not have a negative effect on the cells. The histological data suggest different epidermal layers were formed including the spinous layer, indicating that the keratinocytes reached the final differentiation stage in the epidermis. The low expression of cytokeratin 10 and high expression of cytokeratin 14 suggests the keratinocytes had shifted from a proliferative to differentiated state.

Conclusion
Our findings suggest that the present 3D bioprinted skin model does resemble the native skin epidermis. Using non-animal-derived reagents is a feasible alternative in the creation of alternative models, it enables the development of models that can replace animal models while avoiding the use of animal derived components. Our 3D bioprinted model could be used as a foundation to generate skin models for specific diseases and as an alternative for the use of animal models for cosmetic and drug testing.

keywords: skin, alternative model, 3D bioprinting

31412750205

DIFFERENTIATION POTENTIAL OF CHICKEN EMBRYONIC MESENCHYMAL STEM CELLS AND HUMAN IPS CELLS UNDER INFLUENCE OF CHICKEN LIMB ECTODERMAL JACKET IN OVO

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The recombinant limb technique was developed to study osteo-chondrogenic differentiation and cartilage patterning using stem cells. The method is based on separating the ectoderm from the limb mesenchyme and refilling the ectodermal jacket with the material of interest. Then the engineered limb buds are either reinserted back into the lateral plate mesoderm of the developing chick or placed on the chorioallantoic membrane (CAM). The CAM is an extra-embryonic highly vascularized membrane that is formed from the infusion of mesodermal layers of the chorion and the allantois. Subsequently, tissue development is followed over several days. We use the recombinant limb technique to successfully generate an organized structure from recombinant limbs of chicken mesenchymal cells and human IPS cell aggregates by incorporating a chicken ectodermal jacket. The mesenchymal stem cells were isolated from the limb bud mesenchyme of chicken embryos at HH stages 19-23 and then inserted into the chicken ectodermal jacket collected of the same stage embryos. For human-chick recombinant limbs, the undifferentiated human IPS cells were inserted in chicken ectodermal jacket stage 23 and the recombinant limbs were placed on the CAM.

The growing limbs were characterized using histological staining and in situ hybridization and immunochemistry methods to monitor the efficiency of the ectopic limb development.

keywords: limb development, chondrogenesis, IPS

52354559848

FINITE-ELEMENT AIDED DESIGN OF SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Computer modelling is applied to a wide range of engineering applications to predict the outcome of a physical system. In bioengineering, additive manufacturing (AM) technologies and computer modelling can be placed side by side to fabricate tissue engineering (TE) scaffolds and to study their behaviour under certain circumstances (e.g., the effect of shape, size, and porosity on mechanical properties of a TE scaffold under given loads).
This work aimed to prepare an efficient numerical tool for designing bone TE scaffolds. The behaviour of scaffold structures was studied by varying the pore size and the degree of porosity (pores of diameter between 500 μm and 2 mm, in a range between 60% to 80% of porosity, with uniform and gradient porosities). To find a reliable model, the results of FE analysis were validated against the experimental data.
All designed porous structures were fabricated with stereolithography (SLA) using elastic photo-curable resin to make the validation process much more efficient. SLA technique has been chosen since it produces extremely accurate and high-resolution prototypes with high speed and fine features.
Fabricated specimens were subjected to morphometrical analysis using micro-CT, then mechanically tested with compression (uniaxial and biaxial) tests. FEA simulations were carried out assuming that all fabricated porous structures are made of material with hyperelastic properties.
The results showed good agreement between the experimental tests and the simulations.
Much effort was put toward studying functionally graded (FG) structures (i.e., structures showing gradients of porosity along at least one material direction) since they offer limitless opportunities to tailor functional and structural properties. From the design point of view, a model has been developed that correlates the internal architecture with the final physical properties of the FG structure.
The obtained data are a starting point for future works. With optimization algorithms, the aim is to obtain the best material to tailor specific mechanical properties (e.g., to maximize bone scaffolds' load-bearing and energy storage properties).

keywords: Finite-element analysis, stereolithography, bone tissue engineering

31412706167

HIGHLY CONCENTRATED COLLAGENS ALLOW 3D BIOPRINTING OF STABLE STRUCTURES WHILE ENABLING MOVEMENTS OF RENAL CELLS

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Introduction: Bioprinting large constructs with a good structural integrity is easy, however, it is significant and biologically relevant to select a material that, in addition to providing structural support, enables cells to proliferate, migrate and rearrange the matrix. Soft materials with high cell affinity, like collagen, have in the past been difficult to use for 3D bioprinting of stable complex structures due to their low viscosity. The structural element is an important part when building up different compartments for the fabrication of complex in vitro models for regenerative medicine applications. However, too stiff materials can hinder the movement of the embedded cells which can affect the function of the tissue. For example, the structure of the kidney proximal tubule is composed of a single-layered epithelium of renal proximal tubule epithelial cells (RPTECs) with an apical–basal polarity with a brush border on the apical side to absorb nutrients and to secrete waste products and xenobiotics. These types of structures need to be formed inside the bioprinted construct by a rearrangement of the extracellular matrix by the cells.

Methodology: Here we present two printable, high concentration collagens from Advanced BioMatrix which were bioprinted into stable grids using CELLINK's BIO X. The collagens Lifeink 200 (35 mg/mL) and Lifeink 220 (70 mg/mL) were mixed with immortalized RPTECs before printing. The viability of the cells was assessed using live/dead fluorescent staining on day 1, 7 and 14 after bioprinting. Samples were also stained using immunohistochemistry (IHC).

Results: The viability of the renal cells was above 70% in both collagens after bioprinting and maintained throughout the culture period. There was a clear cell stretching at day 7 and day 14 in both conditions where the cells stretch in all directions. The similarities between Lifeink 200 and 220 in terms of viability and especially in cell stretching, demonstrated that neither of the high concentration collagens hinder the growth nor movement of the RPTECs. With IHC we detected that the cells were allowed to migrate to the boarders of the construct but also lined the natural voids inside both bioinks.

Conclusion: This work highlights the possibility of bioprinting stable structures using collagens that also enable the RPTECs to remodel and move through the matrix. This allows for the fabrication of more complex and representable in vitro kidney models.

keywords: Printable collagen, In vitro kidney model, 3D bioprinting,

62825414586

HUMAN AIRWAY EPITHELIAL CELLS CULTURES FOR TISSUE ENGINEERING APPLICATIONS

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Introduction. Emerging as one of the major causes of disability and death, respiratory diseases affect millions of people worldwide.
To date, despite numerous efforts and clinical attempts, there are no effective treatments to repair long-segment defects, and a standard procedure is still missing.
Among the ventured clinical applications, Tissue Engineering (TE) approaches hold the potential to address the main barriers related to surgical-based procedures, which were all negatively affected by the absence of an integer functional epithelium. A self-renewing well-differentiated epithelium is instrumental for all the physiological functions of the respiratory system, avoiding infections and granulation tissue formation.
In TE strategies, autologous adult airway epithelial cells would be the most appropriate cellular source for restoring the airway epithelium. However, many difficulties have been met during their expansion, and these cells were described as effectively able to divide for a very limited number of passages before losing their differentiative potential, thus being considered an unsuitable cell source for TE approaches.
In the present study, we tested the ability of a culture system, largely used for the clinical expansion of different epithelial tissues, to safely and effectively maintain the long-term proliferative and differentiative potential of airway epithelial cells. Moreover, we established quality controls to be adopted in each step aimed at developing a successful TE construct.

Methodology. Human primary epithelial cells derived from the trachea and the bronchi of different donors were isolated and expanded through a clinical-grade culture condition. During the expansion process, reproducible quality controls were set up to verify i) the quality of the cells extracted from the biopsy in terms of tissue-regenerative properties, ii) the maintenance of the cellular identity, iii) the expression of specific markers for the identification of the different cell types, and iv) the tissue integrity, while single cells assays allowed the identification of stem cells within the cultures.
Finally, to obtain a terminally differentiated epithelium able to mimic the in vivo condition, airlifted cultured were developed.

Results. Epithelial cells extracted from human tracheal and bronchial biopsies were effectively cultured for several passages until replicative senescence, proving the capability of the adopted cultured system to sustain the proliferation of airway epithelial cells. Systematic characterization of cultures during serial passages highlighted the maintenance of differentiative potential throughout the expansion process, expressed as the ability of epithelial cells to differentiate into the main airway cellular types, while the expression of tissue-integrity markers attested the reconstruction of an integer epithelium.
Moreover, the establishment of an air-lifted culture mimicking the in vivo condition allowed to obtain a terminally differentiated airway epithelium expressing also ciliated cells.
Finally, single-cell assays and stem-proliferative markers expression detected the presence of stem cells within the cultures, mandatory for any long-term resolutive TE therapy aimed at restoring the airway district.

Conclusion. The proposed clinically-validated culture system succeeded in establishing a safe and effective expansion of airway epithelial stem cells, allowing the set-up of quality controls and paving the way towards the development of future successful TE applications.

keywords: airway, tissue-engineering, stem cells, respiratory epithelium

62825456567

NOVEL 3D-PRINTED CELL CULTURE INSERTS FOR ADVANCED IN VITRO SKIN REGENERATION

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In skin research, 2D monolayer in vitro models provide only limited fields of application as they do not sufficiently mimic physiological properties. Since in the spirit of 3R's (Replacement, Reduction, and Refinement) animal models are to be avoided, new approaches like 3D skin equivalents (SE) are needed to close the in vitro/in vivo gap. Cell culture inserts to produce SE are commercially available, however, these inserts are expensive and limited regarding the experimental setup.
The aim of this study was to design and produce novel cell culture inserts fabricated on commercially available 3D-printers to generate full thickness human skin equivalents. A computer-aided design model was realized with extrusion-based 3D printing of polylactic acid filament. In consecutive steps the design of the inserts was improved and the feasibility confirmed in cell culture experiments. Cytotoxic effects of the final product were excluded by testing the inserts according to ISO-norm procedures. Finally, to verify usability, full thickness human skin equivalents were created on collagen/fibrin dermal scaffolds which were placed in the inserts. Histologic analysis proved the comparable quality of the constructs compared to commercially available products. In conclusion, here we demonstrate an efficient and cost-effective alternative to produce 3D-printed inserts for the generation of skin equivalents. The system is feasible with common 3D printers and allows high flexibility in the choice of printing materials, membrane materials as well as of shape and size. It is compatible with commonly used cell culture plates and easy to handle. Further studies will be performed with skin equvalents based on the presented technology.

keywords: tissue culture, skin equivalents, 3D-printing, inserts, cell culture inserts

83767289109

OBTAINING OF BIFUNCTIONAL FUSION PROTEINS BASED ON HUMAN INTERLEUKIN 7 AND THEIR APPLICATION FOR BIOMEDICAL RESEARCH

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Interleukin 7 (IL-7) is an immune cytokine that plays a critical role in the maturation and homeostasis of T and B-lymphocytes. The absence of IL-7 significantly affects the proliferation of early lymphoid progenitor cells and leads to the development of severe combined immunodeficiency syndrome. To date, there are active studies of recombinant IL-7 as a means of restoring the immune system of people who have undergone bone marrow transplantation, highly active antiretroviral therapy and chemotherapy. New experimental data are concentrated on the application of IL-7 in the treatment of COVID-19 and as an adjuvant in during vaccination process. IL-7 is widely studied as a therapeutic agent for various immune disorders, that why biomarkers related to immune cell responses to IL-7 that can be used to monitor immune status, individual pharmacological responses to IL-7 treatment, are needed. IL-7 activity can be tested using peripheral blood mononuclear cells (PBMCs) from different donors. The individual response of human PBMCs to IL-7 is valuable prognostic information about the state of the immune system. This information is useful for further large-scale study of the response of T-cell subgroups to IL-7 therapy. This investigations requires highly purified IL-7. The work aimed to obtain rhIL-7 in Escherichia coli and allow protein renaturation with further testing of peripheral blood mononuclear cells (PBMCs) responsiveness to highly purified cytokine. The DNA sequence encoding human IL-7 was subcloned into a pET24a(+) expression vector under control of the T7 promoter and upstream of the vector-derived 6xHis-tag. pET24-hIL7 was transformed into E. coli, protein synthesis was induced with the auto-induction protocol. Immobilized metal affinity chromatography was used for the purification of solubilized protein. Subsequent refolding protocols of rhIL7-His were developed using dilution, gel filtration, on-column refolding, and dialysis strategies. The highest renaturation efficiency of rhIL7-His was obtained in the dialysis method. Gel filtration and refolding on metal affinity sorbent showed comparable lower efficiencies. The activity of rhIL7-His renaturated by dialysis and gel filtration was tested on PBMSc. The response of phytohemagglutinin activated lymphocytes of human peripheral blood was measured in the MTT test. Analysis shows the comparable activity of standard rhIL7 and rhIL7-His renaturated by dialysis. It can be concluded that after dialysis the rhIL7-His protein is obtained in a fully functional form. The study revealed that responsiveness to IL-7 is various for the PBMCs originated from different individuals. Our research assumes that the personalized responsiveness of human PBMCs to IL-7 may be considered as valuable prognostic information about the immune system state and the T cells response to IL-7-based therapies.

keywords: Interleukin 7, protein refolding, immune system state

73296328506

TNFA AND SIRT1 MODULATION AFFECTS BIOENERGETICS AND CHONDROGENIC CAPACITY OF MESENCHYMAL STEM CELLS

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Mesenchymal stem/stromal cells (MSCs) are promising for cell-based cartilage tissue engineering strategies as they can differentiate into the chondrogenic lineage. Although the inflammatory environment often present in the diseased joint can negatively influence cartilage MSC differentiation and cartilage maintenance, we previously demonstrated that the administration of the inflammatory factor TNFα enhanced chondrogenesis of MSC when added prior chondrogenic induction. Interestingly, the master regulator of cellular metabolism sirtuin1 (SIRT1) has also been reported to enhance chondrogenesis in MSC, but it can be inhibited by TNFα. Here we aimed to investigate the combinatory effect of the TNF-α treatment with a SIRT1 activator, in order to evaluate the ability of this co-treatment to further enhance chondrogenic differentiation of MSC. Our hypothesis is that the inhibition of SIRT1 is a potential side effect of the TNFα treatment, and it can be reverted by actively keeping high levels of SIRT1 using a specific activator.
Human MSCs (N=5) were isolated and expanded for 2 passages in a 10% fetal calf serum containing media supplemented with 1 ng/ml FGF-2 (basic expansion media). After, cells were further expanded for one additional passage (4-5 days) divided in 4 conditions consisting in the basic expansion media supplemented with (1) vehicle; (2) 50 ng/ml TNFα; (3) 1 μM SRT1720, a SIRT1 activator; (4) 50 ng/ml TNFα + 1 μM SRT1720. Concentration of TNFα was based on our previous publication, and concentration of SRT1720 was based on a screening evaluating specificity and toxicity of the compound. Chondrogenesis was induced by 3D pellet cultures using a TGFβ-based media for 21 days (same protocol for all the expansion conditions), and it was evaluated by (immune)histochemistry (Collagen type-2 and thionine for glycosaminoglycans) and RT-PCR (AGN and COL2A1). Prior chondrogenesis, monolayer cells were evaluated for bioenergetics parameters (XF-Seahorse Analyzer, Bioscience) and β-catenin pathway activation (western blot).
After chondrogenic induction, TNFα-expanded cells resulted to be the most chondrogenic, while the TNFα + SRT1720-expanded cells were the least chondrogenic, often with no visible staining for either Collagen type-2 or thionine. Interestingly, an in-depth bioenergetics analysis revealed that the MSC stimulated with TNFα + SRT1720 had the higher levels of basal and maximal oxygen consumption rate (OCR), and of the extracellular acidification rate (ECAR), indicating an overall metabolic enhancement. Analysis of the WNT/β-catenin signaling on those cells, on one hand confirmed our previous observation that TNFα-stimulated cells have enhanced β-catenin activity, on the other hand it showed that further administration of SRT1720 on TNFα-stimulated MSC abolished this effect.
Contrary to our initial hypothesis, co-stimulation with TNFα and the SIRT1 activator SRT1720 during expansion is detrimental for chondrogenic capacity of MSC. This is possibly due to an hyper- metabolic activation in these cells and a negative effect on the WNT signaling pathway, parameters known to sustain MSC differentiation.

keywords: Chondrogenesis, cellular metabolism, inflammation, TNFa, SIRT1

PS47
New insights underlying mesenchymal stem cell-mediated bone regeneration

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A NOVEL BIOMIMETIC KNEE JOINT BIOREACTOR FOR THE IN VITRO REGENERATION OF OSTEOCHONDRAL LESIONS

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The biomedical sector is experiencing a technological transformation in its attempt to solve current and next future global health issues. The scarcity of organ donors in a growing elderly population accentuates the need to generate new strategies based on the use of cutting-edge technologies for regenerative medicine.
In recent years, the emergence of 3D bioprinting has allowed great advances in tissue engineering, making feasible the automatized and controlled ex vivo generation of bioartificial substitutes with a certain degree of complexity. The controlled application of biomimetic biochemical, physical and mechanical stimuli of a given tissue within custom-designed bioreactors for each bioprinting application is critical to promote the maturation of the 3D construct towards a functional tissue with clinical applications. All these stimuli are well-acknowledged to be critical players in determining cellular fate and functions during tissue formation, homeostasis and disease. However, there are so far no bioreactors available in the market for tissue engineering. Technical complexity and economic costs are main factors that have limited their development by research institutions and companies.
REGEMAT 3D has developed a novel bioreactor that mimics the anatomy and physiology of the human knee (BMAP Knee) and promotes cartilage regenerative processes under a controlled microenvironment. The bioreactor maintains the temperature, the O2 and CO2 concentrations of the cell culture within the physiological range and can apply typical movements of the knee (flexo-extension, compression and rotation) in patient-specific knee joint models obtained by 3D bioprinting.
In a pilot study, reconstruction of the human knee joint was performed through 3D printing from MRI data of a patient with osteochondral lesions in lateral and medial condyles of the femur. Polylactic acid was employed for the printing of femur and tibia while menisci and osteochondral lesions where printed with polycaprolactone to provide them with more flexibility. 3D Cell culture was performed within osteochondral lesions using a bioink composed by human adipose tissue-derived mesenchymal stem cells and a hydrogel of collagen type 1. The application of compressive mechanical loads (0.3 Hz, 4h/day, total of 21 days) under physiological conditions of temperature, CO2 y O2 concentrations promoted the activation of genes encoding the SOX9 transcription factor, collagen type 2 (COL2A1) protein and aggrecan (ACAN) proteoglycan, which are the most abundant components of the cartilage extracellular matrix. These results suggest the activation of chondrogenic differentiation the differentiation of mesenchymal cells in the osteochondral lesions in response to mechanical stimuli. In addition, cell viability and proliferation measurements demonstrated the bioreactor's capacity to maintain the scaffolds under appropriate physiological parameters and sterile conditions in the long term, thus validating its use for tissue engineering purposes.

keywords: bioreactor, mesenchymal cells, osteochondral repair

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AMINO ACID SUPPLEMENTATION ENHANCES HBMSCS OSTEOGENIC CAPACITIES

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Introduction
Around 10% of long bone fractures display inadequate bone regeneration, leading to delayed- or non-unions. Amino acids have shown to play important roles in bone regeneration. In particular, citrulline has revealed itself as an enhancer of bone regeneration in murine bone defect healing models. Oral citrulline supplementation stimulated callus formation and lead to a balanced regulation of inflammation, resulting in a shorter inflammatory phase after fracture, thereby contributing to enhanced bone regeneration. However, the exact cellular mechanisms behind these enhanced regenerative capacities are unknown. The aim of this study was therefore to determine whether citrulline can enhance the osteogenic capacity of human Bone-marrow Mesenchymal Stem Cells (hBMSCs) as well as MG63 (osteosarcoma) cells.

Methods
For this study, hBMSCs of 5 healthy donors (mean age 59 ± 9; 4 males) and MG63 cells were cultured in osteogenic medium for a period of three weeks. Citrulline was supplemented in the following concentrations (mM): 0, 5, 7.5, 10. Alanine was administered in isocaloric control concentrations. Cellular viability was assessed through presto blue assay, proliferation by DNA quantification, and cytotoxicity through lactate-dehydrogenase assay. For the determination of the osteogenic capacity, ALP activity and alizarin red staining were performed, combined with qPCR for osteogenic marker genes. Measurements were performed at days 1, 3, 7, 14, and 21.

Results
For hBMSCs, the greatest effect of citrulline supplementation was observed at day 21, at a concentration of 7.5 mM. ALP activity and cellular viability were enhanced increasingly over time upon citrulline supplementation. Contrarily, cellular viability and ALP activity in the alanine supplemented and blank control groups remained constant per time point. Additionally, for all timepoints, citrulline supplemented hBMSCs showed enhanced cellular proliferation defined by DNA quantification as compared to the blank medium control. This effect was most evident for all time points at a concentration of 7.5 mM. Overall, alanine supplementation did not enhance cellular proliferation compared to the blank medium control.
For the MG63 cells, ALP activity was minimal, and no differences were observed between the two amino acid treated groups. Cellular proliferation and viability also did not show significant differences among the two amino acid treated groups.

Discussion
This study revealed potential cellular mechanisms behind enhanced bone regeneration upon supplementation of specific amino acids. Citrulline supplementation induced an increased

expression of the early osteogenic differentiation marker ALP, and enhanced cellular proliferation in hBMSCs, suggesting that these cells contribute to the stimulatory effect of citrulline on bone regeneration. This study will further focus on the effect of citrulline on osteogenic capacities regarding mineralization and the expression of osteogenic marker genes runx2, osterix, osteocalcin, osteopontin, collagen type 1, and iNOS, and analysing the possible cytotoxic effect of citrulline supplementation.

keywords: bone regeneration, hBMSCs, osteogenic differentiation, amino acids

73296330486

DIFFERENCES IN PERIODONTAL LIGAMENT STEM CELLS FROM MAXILLA AND MANDIBLE

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Introduction:
Diseases of the periodontium start in general with the recession of the periodontal ligament (PDL) and leads to loosening of the tooth within the alveolar bone. The cementum and periodontal ligament play important roles in physiological tooth function. Therefore, cementum and periodontal ligament regeneration are critical for periodontal regenerative therapy. Decades of clinical experiences indicate, that velocity and efficiency of wound healing and bone remodeling in alveolar bone and PDL is in part dependent on the exact location of the wound. In the maxilla, the upper jaw, these processes are generally faster and more efficient than in the mandible, the lower jaw. Alveolar bone differs in composition, with 23% bone marrow and 46% lamellar bone in the upper jaw, and 16% bone marrow and 63% lamellar bone in the lower jaw, which may affect the recruitment of stem cells towards a wound. The PDL hosts endogenous stem cells, which were shown to differentiate towards osteoblast and cementoblasts in 1976. Since 2004, specific PDL cells can be isolated from extracted third molars, demonstrating self-renewal and differentiation capacity towards several mesodermal cell fates and therefor being referred to as stem cells. PDL cells in particular mesenchymal stem cells (MSC) have been extensively studied with respect to bone and tissue replacement strategies. The characterization of PDL cells from the upper and lower jaw compared to MSC will identify similarities and differences in cellular composition and unravel molecular pathways involved in differentially regulated wound healing in mandible and maxilla.

Methods:
PDL cells were isolated from extracted third molars of young and healthy patients. Human tissue donations were received with informed consent. All cells were cultured with DMEM high glucose, 10% FCS and 50mg/l ascorbic acid in a 20% O_2 and 5% CO_2 humidified atmosphere at 37°C. Only patients with extracted teeth from both upper and lower jaws were considered for this study, to have a direct comparison between maxilla and mandible. PDL cells were compared to MSC isolated from the spongiosa of femoral heads. Surface characterization for stem cell (CD34-, CD45-, CD73+, CD90+ and 105+) and PDL specific marker were performed for both cell type. Proliferation rate and differentiation potential towards adipocytes, osteoblasts and chondrocytes of PDL cells from the upper and lower jaw was investigated. Wound healing assays were performed by scratch assay and migration of both cell types was evaluated by Boyden Chamber assays.

Results:
First results showed that PDL cells, isolated from upper and lower jaw, were positive for CD73, CD90 and CD105 but negative for CD34 and CD45. PDL cells from the upper jaw have a higher proliferation rate and differentiation potential when compared to PDL cells that were isolated from the lower jaw.

Conclusion:
The wound healing and bone remodeling processes are more efficient in the upper jaw, than

the lower jaw. However, the underlying regulatory mechanism is unclear. In this study, we attempted to identify regulatory genes involved in periodontal cell differentiation and clarify the differentiation mechanism for effective periodontal regenerative therapy.

keywords: dental, tissue engineering, stem cells

73296315448

FUNCTIONAL CHARACTERIZATION OF HUMAN BONE MARROW STROMAL CELLS IN VIVO WITH INCREASED THROUGHPUT

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Introduction:
The human bone marrow (BM) is home to Hematopoietic Stem Cells (HSCs) and Bone Marrow Stromal Cells (hBMSCs) which are known to contain skeletal stem cell populations. Whereas the HSC compartment is well characterized, the identity and function of the hBMSC populations remain obscure and requires thorough investigation. A better understanding of the BM and the accommodated cells is crucial as changes in the microenvironment homeostasis may cause severe diseases including leukemia. Recent advances in single cell analysis featured hBMSCs as a heterogeneous cell population with only a small fraction of cells having the potential for multilineage differentiation and long-term self-renewal [1]. To unveil the true fate of hBMSCs we perform cell implantations in vivo to reliably verify their function in the complex native environment.

Methodology:
To reveal the function and hierarchical organization of distinct hBMSC subpopulations we propose to engineer a multiplexing screening device for efficient in vivo testing. Additionally, using transglutaminase crosslinked poly(ethylene glycol) (PEG) hydrogels we are establishing robust microenvironmental conditions for the osteo-, chondro- and adipogenic differentiation of hBMSCs. Then, we will encapsulate prospectively isolated hBMSC populations in defined microenvironments and place them in the implantable multiplexing device. Finally, multiplexing devices will be implanted in subcutaneous pouches of immune-deficient mice and used to assess the in vivo differentiation capacity of candidate subpopulations of hBMSCs.

Results:
First experiments dedicated to minimize the number of required hBMSCs and increasing the number of test conditions have revealed in vivo differentiation in an osteogenic microenvironment and formation of small bone ossicles containing a hematopoietic niche within the multiplexing device.

Conclusion:
In this project, we develop a multiplexing platform to screen hBMSC behavior in vivo in a higher throughput manner. We will optimize the designs for multiplexed testing of health and disease-related low-abundant hBMSC subpopulations, requiring minimal cell numbers and tiny hydrogel volumes. The results of this project will constitute an important foundation to study human BM stromal hierarchy and elucidate the functional role of individual hBMSC subpopulations.

References:
[1] Chan, C. K. et al., Cell, 175(1), 43-56 (2018).

keywords: Bone marrow stromal cell, bone marrow niche, poly(ethylene glycol), bone organoid

62825424404

LIQUIFIED MICROCAPSULES: A VERSTILE PLATFORM TO APPLY HIGH HYDROSTATIC PRESSURE TO HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FOR OSTEOGENIC DIFFERENTIATION

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Bone is constantly exposed to a range of macro-scale loading which creates a complex mechanical microenvironment for its resident cells. One such mechanical stress generated is hydrostatic pressure (HP) which plays an important role in cell function and fate determination. Although HP is a constant mechanical cue for bone resident cells, little is known about the effect of this external stimuli in a 3D microenvironment. Inspired by native bone mechanical microenvironment, this research studied for the first time the effect of different ranges of cyclic HP on human adipose-derived mesenchymal stem cells (hASCs) encapsulated in a 3D liquefied microcapsule. In the proposed system, while encapsulated hASCs were free in a liquid environment, surface functionalized microparticles were provided as cell attachment sites. In the first step of the study, different ranges of HP (10-250 MPa) were applied to the hASCs for 10 minutes, to find the maximum magnitude that cells could survive. According to the results, 50 MPa was the highest applicable pressure without jeopardizing cellular viability. Then, cyclic HP (6 cycles of 10 minutes) was applied to the hASCs encapsulated in microcapsules in a low (5 MPa) or a high (50 MPa) magnitude.
The electrospraying technique was employed to produce alginate microgels encapsulating hASCs and microparticles in a calcium chloride bath [1]. Using alginate microgels as templates, a multilayered membrane made of poly(L-lysine), chitosan, and alginate polyelectrolytes were produced via layer-by-layer assembly technology (n=12-layers). After a mild core liquefaction process, liquefied microcapsules were cultured in basal (BAS) or osteogenic (OST) media up to 21 days. Taking advantage of the liquefied core environment of microcapsules, hASCs were exposed to cyclic HP at 5 or 50 MPa magnitudes 3 times/week.
Biological tests including MTS and live-dead assays indicated that hASCs remained viable up to 21 days of culture in all tested conditions. The fluorescence staining of F-actin filaments demonstrated a noticeable increase in cell-cell interactions and network formation of hASCs in the pressurized groups, compared to the non-pressurized group. Being this phenomenon more pronounced in OST condition, the observation confirmed by fluorescent staining of vinculin. Results showed that vinculin distribution increased in response to pressurization, specifically in OST group. More interestingly, a significantly higher alkaline phosphatase activity was detected in 50 MPa group. Furthermore, a greater staining of osteopontin, and hydroxyapatite markers was observed in 50 MPa/OST group.
Overall, this study demonstrated that the proposed liquefied encapsulation system holds great potential as an effective platform for studying the impact of various magnitudes of HP for numerous differentiation purposes. Moreover, results revealed that the beneficial effect of HP for osteogenic differentiation is magnitude dependent. Finally, the highest differentiation effect was observed when both biochemical and mechanical cues were combined (50 MPa, OST).

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Acknowledgements
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Inspired by native bone mechanical microenvironment, this research studied for the first time the effect of different ranges of cyclic HP on human adipose-derived mesenchymal stem cells (hASCs) encapsulated in a 3D liquefied microcapsule.

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keywords: Mechanical stimulation, Liquefied microcapsules, Hydrostatic pressure, Mesenchymal stem cells, Osteogenic differentiation

PS48
Next Generation Biomaterials of Stem Cell Culture and Differentiation for Stem Cell Therapy

52354532924

ENGINEERING BIOMIMETIC HYDROGEL SCAFFOLDS FOR TISSUE REGENERATION

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Introduction
Everyday thousands of people of all ages across the world are admitted to hospitals because of the severe injuries or malfunction of some vital organs [1]. Organ or tissue transplantation is a standard therapy to treat these patients. Irony of fact that many of these people will die due to the paucity of donor organs and high processing cost involved in organ transplantation [2]. To meet this global clinical need, tissue engineering has emerged to regenerate injured or diseased tissues and organs. Biomaterials serve as scaffolds to support cells as well as to guide them towards specific tissue construct [1]. Recently, hydrogels - a crosslinked three-dimensional (3D) polymeric network with elegant ability to mimic extracellular matrix functionalities - have evolved as a potential scaffold for controlling/guiding cell fate towards a specific tissue/organ [3]. However, it is still a critical engineering challenge to design clinically relevant hydrogel scaffolds in a congenial and sustainable approach. Herein, we develop a versatile, customizable, and scalable synthesis protocol of hydrogel scaffolds with physiologically relevant features for therapeutic applications.

Methodology
We successfully developed a green synthesis approach, maintaining an engineering mindset, to formulate a set of gelatin-based multicomponent hybrid hydrogels with 3D porous structural stability, tunable biomimetic mechanical properties, controllable degradability, electrical conductivity, efficient sterilizability, and biocompatibility [4]. The novel eco-friendly hydrogel synthesis protocol successively involved four steps: (i) liquid-phase crosslinking/grafting, (ii) unidirectional freezing, (iii) freeze-drying, and finally (iv) post-curing. Taking advantage of the reactivity of epoxide-end PEG (act as crosslinking agent) with available various functional groups of gelatin in aqueous environment, the crosslinked network of gelatin-PEG was attained, keeping a constant feed ratio of gelatin to PEG for all formulations.

Results
The developed synthesis strategy offered simplicity, versatility, customizability, sterilizability, biocompatibility and scalability - a combo of some important features favoring clinical translation of biomaterials. All hybrid hydrogels showed highly interconnected open porous structures suitable for tissue engineering applications. The hydrogels showed constituent- and concentration-dependent anisotropy. Mechanically, all hydrogels showed robust stability, J-shaped stress-strain curve, excellent shape recoverability, strong fatigue resistance and stress relaxing behavior. Biological experiment with human bone marrow mesenchymal stromal cells revealed that hydrogels were biocompatible, and their compositions and dynamic mechanical properties were suitable to support stem cell proliferation, as well as osteogenic and chondrogenic differentiation. Both variation in compositions and stress relaxation behavior of hydrogels were found to guide stem cells towards different level of matured osteogenesis and stable chondrogenesis.

Conclusion
The developed robust synthesis strategy offers preparation of next generation hydrogel scaffolds with tissue-like mechanics and morphologies, tunable degradation, greater customizability in composition and architecture, and biocompatibility for a wide range of tissue engineering applications, including nerve to bone tissue regeneration.

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keywords: Biopolymers, hybrid hydrogels, stress relaxation, stem cells, differentiation

41883668888

GRAPHENE OXIDE AS A CHONDROINDUCTIVE BIOMATERIAL FOR ARTICULAR CARTILAGE REGENERATION

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Articular cartilage has a poor capacity for self-repair, consequently, defects are one of the major causes of immobility and poor quality of life for millions of individuals worldwide. Damage to articular cartilage can be caused by trauma, ageing, genetic factors and degenerative diseases such as osteoarthritis and is often irreversible without surgical intervention. The current clinically approved therapies for cartilage repair rely on the use of autologous chondrocytes and can be limited by chondrocyte expansion and generation of fibrous repair tissues with inferior mechanical properties. This has led to the emergence of alternative cartilage tissue engineering (CTE) as a strategy to regenerate long-lasting functional tissues.

Human pluripotent stem cells (hPSCs) show exciting potential as an alternative cell source for cartilage regeneration. This is due to their capacity for unlimited self-renewal and ability to differentiate into almost all cell types within the body, thus forming an unlimited supply of cells for regenerative therapies. Signalling from the TGF-β growth factor family plays a critical role in regulating chondrogenic differentiation and maintenance of articular cartilage tissues. However, growth factor-driven chondrogenic differentiation is limited by the intrinsic properties of growth factor peptides such as short half-life, rapid denaturation in vivo and high costs[1].

Graphene oxide (GO) is a 2D carbon nanomaterial that has gained attention worldwide due to its extraordinary physiochemical properties. The advantage of GO over other nanomaterials is its ability to act as a multifunctional bioactive moiety, stemming from its unique 2D structure and rich surface chemistry which facilitates functionalisation. GO has been shown to improve chondrogenesis following the absorption of proteins and growth factors onto the surface of GO flakes or through improving mechanical properties within hydrogel scaffolds. However few studies have investigated the intrinsic chondroinductive effects of GO alone.

Here we demonstrate that GO alone is able to stimulate the TGF-β signalling pathway in human chondrocytes (TC28a2) and hPSC derived chondroprogenitors, thus highlighting a potentially new and cost-efficient means of inducing cartilage regeneration. Induction of the canonical TGFB signalling pathway was demonstrated through the use of a TGFβ signalling reporter (SBE-nLUCp) [2] and further validated by pSMAD2 immunoblotting and analysis of TGFB response genes via RT-QPCR. Exploiting the unique intrinsic fluorescence of our GO enabled robust analysis of interactions between GO and single chondrocytes through confocal live-cell imaging using the method developed by Vranic et al [3]. This facilitated an in-depth understanding of the mechanisms by which GO increases TGFB signalling activity.

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doi: 10.1021/acsnano.7b07734.

keywords: Graphene Oxide, Cartilage, TGF-β, Pluripotent stem cells

20941834206

IDENTIFICATION OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS POTENTIALLY USEFUL FOR THE GENERATION OF BIOARTIFICIAL TISSUES BY TISSUE ENGINEERING

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Introduction: In the last decade, the interest in understanding perinatal tissues, including the human umbilical cord, have exponentially increased due to the plasticity, differentiation, and immune modulatory properties of these tissues1,2. Advances in the comprehension of the biology of human umbilical cord and their cells, will contribute to establish their potential use in tissue engineering and advanced therapies. The aim of this work is to characterize the human umbilical cord and the mesenchymal stem cells found in this structure as potential cell source in tissue engineering.

Methodology: Human umbilical cords (hUCs) were obtained from full-term newborns delivered by cesarean section. Transversal sections of the hUCs were obtained, fixed in 4% formaldehyde washed, dehydrated, cleared, embedded in paraffin, and finally stained with hematoxylin and eosin (HE) for histological analysis. Histochemical and immunohistochemical analyses were carried out to evaluate the expression of key biological markers of MSC (CD105, CD90 and CD73) and extracellular matrix (ECM) components. In each sample, four regional areas of the umbilical cord were analyzed: intervascular zone (IV), perivascular (PV), subamnioblastic (SAM) and Wharton jelly zone (WH). The staining intensity and the percentage of cells showing positive signal for each marker were quantified in each sample and each zone.

Results: Our results demonstrate that MSC residing in the hUC express the typical markers of MSC CD90, CD105 and CD73, although expression varied among the four regions of the hUC. In addition, all zones were positive for collagen and proteoglycans, suggesting that these cells may play a role in remodeling the ECM of the hUC. However, regional differences were found, suggesting that cells corresponding to specific regions may be more suitable for tissue engineering purposes.

Conclusions: These results support the use of MSC derived from the hUC as cells with potential

to synthetize relevant fibrillar and non-fibrillar ECM components without losing the expression of the key MSC markers. These results open the door to the use of specific cell types isolated from IV, PV, SAM or WH regions with increased potential to synthetize definite ECM molecules for the generation of bioengineered substitutes of the human skin, oral mucosa, palate, cornea and other tissues by tissue engineering.

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keywords: Human Umbilical Cord, MSC, Tissue Engineering

94238119105

OPTIMIZATION OF CELL CULTURE PROTOCOLS USING 3D PLATFORMS FOR USE IN ORAL MUCOSA AND CORNEA TISSUE ENGINEERING

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Introduction: Cell culturing of human cells used in oral mucosa and cornea tissue engineering can be optimized by using three-dimensional cell culture platforms1. In general, these systems comprise an extracellular matrix where cells can be cultured in a 3D spatial microenvironment able to simulate the physiological conditions in which cells are found in vivo, including cell-cell and cell-extracellular matrix interactions2. These interactions are essential for mesenchymal stem cells development, whose therapeutic effect is directly related to their paracrine activity. The aim of this study is to evaluate the impact of these systems on cell behaviour of human umbilical cord Wharton's Jelly Stem Cells (hWJSC).

Methodology: hWJSC were isolated and cultured in 2D conditions (control cells cultured in routine culture medium) and in a 3D system consisting of a part of culture medium and a part of an extracellular matrix (ECM) generated in the laboratory. Different study groups containing increasing concentrations of ECM (25%, 50%, 75% and 100%) were studied. Cells were cultured in each condition were evaluated after 7 days of culture at 37ºC with 5% CO2. Then, total RNA was isolated from each experimental condition and retrotranscribed to cDNA. Real-time qRT-PCR was performed, and the relative expression levels of several genes involved in apoptotic cell death and cell differentiation was determined.

Results: First, we found that hWJSC were able to grow and proliferate in the 3D platform, and their morphology and histological profile was similar to cells cultured in 2D systems. Then, the use of real-time qRT-PCR revealed that the gene expression profile of both types of cells was very similar, with very low expression of caspases and other genes related to cell death by apoptosis at the mRNA level. In addition, the highest concentrations of ECM used in the 3D systems was associated to an increment in certain genes related to cell undifferentiation.

Conclusions: In general, these results suggest that the 3D platform was able to support cell growth and proliferation, and that hWJSC cultured in these systems kept high levels of cell viability and cells maintained their undifferentiated status. These results support the use of ECM-based 3D systems for culturing hWJSC for oral mucosa and cornea tissue engineering purposes.

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keywords: Wharton Jelly Stem Cells, Cell Culture, Three-dimensional

31412701644

OSTEOGENIC DIFFERENTIATED ADIPOSE-DERIVED STEM CELLS CREATE AN IN VITRO BONE MODEL INSIDE MICROFLUIDIC PLATFORMS

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INTRODUCTION
Mesenchymal stem cells have contributed to the continuous progress of tissue engineering and regenerative medicine. Among different mesenchymal stem origins, the ones derived from adipose tissue possess many advantages including the ease of tissue harvesting, self-renewal potential and rapid population doubling time. Adipose derived stem cells (ADSC) have multipotent ability and they can differentiate into different cell linages: osteoblastic among others. In vitro bone models represent a much needed tool to carry out an initial safety assessment in the study of novel bone regenerative therapies1,2. We hypothesized that 3D bone-on-a-chip models containing ADSC could closely recreate the physiological bone microenvironment. Thus, they might become an intermedium step between the traditional 2D-in vitro and the in vivo experiments to speed up the screening of different therapeutic molecules while saving resources.

METHODOLOGY
In this work we differentiated ADSC during 7 and 14 days and they were used to fabricate in vitro bone models. Undifferentiated ADSC were also assayed as negative control. Those pre-differentiated and undifferentiated cells were embedded in a 3D collagen matrix placed in a microfluidic chip. Microfluidic devices were fabricated out of PDMS as described previously3 and the hydrogel was composed of rat tail collagen Type-I. Cells were seeded at a final concentration of 1.106 ADSC/ml.
Biochemical assays and staining procedures were conducted to study cell type and morphology after 3, 7, 14, 21 days of culture. Osteogenic markers such as Alkaline phosphatase (ALP) activity, calcium mineralization, changes on cell morphology and expression of specific proteins (bone sialoprotein 2 (BSP-2), Dentin matrix acidic phosphoprotein-1 (DMP-1) and osteocalcein (OCN)) were evaluated inside the devices to determine their differentiation potential and the evolution of the culture. To the best of our knowledge, this is the first time a 3D-in vitro bone model from adipose-derived stem cells has been developed.

RESULTS AND CONCLUSIONS
ALP activity, specific protein release and culture morphology evolution show the stem cells osteogenic transition and prove that mature bone cells are obtained at the end of the culture. Thus, fully osteogenic differentiation has been successfully achieved inside the microfluidic device. Collagen hydrogel embedding bone cell niches is able to replicate bone tissue environment in vitro. This fact offers a powerful tool to study, inside a thoroughly controlled atmosphere, bone cell responses against different stimuli.

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keywords: Stem cells, osteogenic differentaition, microfluidics, collagen hydrogel

41883667779

SYNERGISTIC MECHANICS OF COMPOSITE BIOMATERIALS AFFECTS EARLY CELL RESPONSE AND CHONDROGENESIS OF MSCS

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INTRODUCTION
There is a growing interest in biomimetic materials which are capable of recapitulating the native extra-cellular environment and drive/guide cell behaviour. Hyaluronan (HA) and fibrin (FB) are two natural extracellular components that each play a pivotal role in modulating cellular behaviour, such as proliferation, migration and differentiation. Cells are capable of sensing their surrounding mechanical environment and responding via a process known as mechanotranduction. This mechanosensitivity involves cytoskeletal deformation and propagation of mechanical signals throughout the cell which is known to affect cell differentiation. We aim to engineer composite hydrogels made of HA and FB that are characterised by different mechanical properties with the ultimate goal of guiding cell response.

METHODS
HA conjugated with tyramine (Ds 11%) was mixed with FB in mass ratios 1:1, 2:1 and 4:1 keeping a constant [FB] (2mg/ml) and varying [HA]. Rheological properties of the composites were evaluated with frequency sweep and stress ramp tests and compared with those of the individual components. Fluorescent confocal microscopy was used to evaluate fibrous fibrin network structure within the hydrogels. Human Mesenchymal Stromal Cells (hMSCs) were encapsulated in HA/FB hydrogels at 3*106 cells/ml and cultured up to 28 days in standard chondrogenic medium. Cell morphology and the organization of actin cytoskeletal structures were evaluated at 1 and 3 days post encapsulation by phalloidin staining and microscopy. RNA was purified from samples cultured for 28 days and gene expression analyses were performed with RT-qPCR.

RESULTS
1:1 and 2:1 HA/FB composite hydrogels had up to 5-fold higher storage modulus than the sum of the pure components, indicating a synergistic effect of combining HA and FB. This effect disappeared in the condition 4:1 HA/FB whose mechanical properties approximated pure HA. Furthermore, the 1:1 and 2:1 HA/FB composites had similar storage modulus to each other while 4:1 had a higher storage modulus. Confocal microscopy showed fibrous space-spanning network in all composites materials. The morphology of hMSCs cultured in HA/FB hydrogels was different in the different composites. Within 3 days, cells in the 1:1 hydrogel had an elongated morphology with several branches. On the contrary, in the 4:1 condition, cells retained a more spherical phenotype. In all hydrogels, cells expressed typical chondrogenic markers COL2A1, ACAN and SOX9, with a higher expression in hydrogels with higher relative percentage of HA.

DISCUSSION & CONCLUSIONS

By modulating hyaluronan and fibrin relative ratios we were able to engineer composite hydrogels with different mechanical properties. For certain ratios, we observed mechanical reinforcement, possibly linked to a prestressed state of the fibrin network in the presence of higher amounts of hyaluronan. Cells encapsulated in these materials acquired different cytoskeletal conformation within 3 days suggesting that the different HA/FB composites influence early cell response and possibly guide cell differentiation. While all the investigated conditions supported chondrogenic differentiation of MSCs, we could observe a higher expression of chondrogenic markers correspondingly to higher HA percentage in composites materials. This aligns with the expected spherical morphology for chondrocytes which is observed at higher HA concentrations.

keywords: biomaterials, tissue mechanics, cell-matrix interaction, mechanotransduction,

52354521084

THE VOLATILOME OF HUMAN PLURIPOTENT STEM CELLS USING SELECTED ION FLOW TUBE MASS SPECTROMETRY

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Introduction
Human pluripotent stem cells (hPSCs) proliferate indefinitely and produce cells from the three germ layers. Such abilities have tremendous applications in the biomedical field. Large amounts of hPSCs are needed for such purposes, which require prolonged in vitro culture. Unfortunately, long-term culture is associated with genomic and phenotypic changes in hPSCs, and thus, routine characterisation is required to ensure the maintenance of a healthy state1. Furthermore, the presence of hPSCs in cell therapies has severe implications as these cells are associated with a risk of tumorigenicity. Any residual hPSCs must be identified and eliminated before clinical translation. Characterisation and, consequently, identification of hPSCs, can be done by measuring the cells' metabolism (i.e., the concentration of metabolites in a cell). Volatile organic compounds (VOCs) are metabolites that offer information regarding the metabolic activities in cells. A strategy to detect VOCs is selected ion flow tube-mass spectrometry (SIFT-MS). SIFT-MS analyses humid gaseous samples for several compounds simultaneously and in real-time2. Here, we used SIFT-MS to investigate the VOC profile of various hPSC lines to find differences and/or commonalities between the lines.

Methodology
The hPSC lines SHEF-1, SHEF-2 and ZK2012L were cultured in xeno-free conditions. Each line was seeded at a specific split ratio, so confluency at 70-80% would be reached after 3 days in culture. hPSC conditioned media (CM) was collected on day 1 and day 3 post-passaging and transferred into 150mL glass bottles for SIFT-MS measurements. Media-only controls were also created, corresponding to media incubated on vitronectin-coated culture vessels without cells for the equivalent time as the cellular counterparts. The bottles' headspace was purged with dry and sterile air (20% oxygen and 80% nitrogen mixture), and bottles were incubated for 16 hours at 37°C. The gaseous headspace above the cells was then measured and analysed using multiple ion monitoring (MIM) mode with H_3O^+ and NO^+ precursor ions. Samples were measured for approximately 50 seconds. Samples were normalised to 4% water level.

Results
Overall, MIM data showed that the concentration of VOCs generally decreased in hPSC CM collected at day 3 compared to day 1. Additionally, each cell line displayed distinct amounts of individual VOCs. hPSCs were also positive for pluripotent markers (NANOG, OCT4, SSEA-4 and alkaline phosphatase), analysed by immunohistochemistry and flow cytometry, which validated the results observed from SIFT-MS.

Conclusions
The distinct VOC profiles observed for individual hPSC lines may reflect the inherent high variability associated with hPSCs1. Furthermore, these differences were detected without apparent changes in protein expression. Therefore, SIFT-MS can identify smaller changes in hPSCs behaviour that pass unnoticed when using standard characterisation techniques. SIFT-MS represents a non-invasive method that provides a detailed characterisation of hPSCs. This is a significant advantage over current non-invasive methods, like live staining, which are limited

in the information they offer regarding hPSC status. SIFT-MS can be a valuable resource in the monitorisation of hPSCs for cell manufacturing and clinics.

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keywords: Human Pluripotent Stem cells, Exometabolome, Volatile Organic Compounds, Selected Ion Flow Tube-Mass Spectrometry

PS49
Novel strategies to assess cellular response to biomaterials

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A 3D TENDON BIOMIMETIC SCAFFOLD WITH POTENTIATED BIOLOGICAL PERFORMANCE ON AMNIOTIC EPITHELIAL STEM CELLS FOR TENDON TISSUE ENGINEERING APPLICATIONS

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Introduction: Advanced strategies in tendon Tissue Engineering might overcome the unsatisfactory results of conventional treatments for tendinopathies [1]. It aims at designing 3D tendon biomimetic scaffolds with adequate physical, mechanical, and biological properties of the native tissue. In this context, 3D tendon biomimetic poly(lactide-co-glycolic) acid (PLGA) scaffolds with highly aligned fibers were fabricated and engineered with Amniotic Epithelial Stem Cells (AECs), to verify their teno-regenerative and immunomodulatory potential.

Methods: Electrospinning technique has been used to produce PLGA fleeces with highly aligned fibers. 3D scaffolds, obtained by wrapping manually the fabricated PLGA fleeces, were characterized for their ultrastructure and mechanical properties. The teno- and immuno-inductive potentials of PLGA fleeces and 3D scaffolds have been assessed on AECs by analyzing YAP, a mechanotransducer protein, TNMD protein, a mature tendon marker, and tendon-related (SCX, COL1, and TNMD) and interleukins (IL-10 and IL-12) gene expressions after 48h and 7d culture. AECs cultivated on Petri dishes were used as control (CTR).

Results: The fabricated PLGA 3D scaffolds mimic the structure of native tendons, characterized by high fiber alignment and significantly higher biomechanical properties compared to PLGA fleeces ($p<0.05$). AECs engineered within PLGA fleeces and 3D scaffolds changed their cobble-stone morphology, acquiring a spindle tenocyte-like one already after 48h culture, confirmed by the decrease in cell nuclei aspect ratio with significantly lower values for 3D scaffolds ($p<0.05$). The teno-inductive potential of the fabricated materials was confirmed by analyzing TNMD protein expression and tendon-related genes. TNMD was already expressed after 48h culture within the cytoplasm of AECs engineered within PLGA fleeces and 3D scaffolds. Moreover, all tendon markers were significantly upregulated by the cells seeded on both PLGA materials with respect to CTR ($p<0.05$), especially after 7d culture ($p<0.05$). The immuno-inductive potential of PLGA materials on AECs was confirmed by the significant expression of IL-10, an anti-inflammatory cytokine, with boosted upregulation for 3D scaffolds at 7d ($p<0.05$). Instead, the

expression of IL-12, a pro-inflammatory cytokine, was maintained to its basal levels within 3D scaffolds. The IL-10/IL-12 ratio was especially in favor of 3D scaffolds ($p<0.05$). The effect of fiber topography on AECs' biology was assessed by analyzing YAP protein expression. Differently to CTR where YAP was localized within cells' cytoplasm, PLGA fleeces and 3D scaffolds induced YAP localization within AECs' nuclei with significantly enhanced activation on 3D scaffolds compared to fleeces ($p<0.05$). Indeed, 3D scaffolds exerted an enhanced activation of YAP mechanotransducer on AECs'.
Conclusions: The obtained results demonstrated that the fabricated 3D scaffolds mimic native tendon tissue ultrastructure and biomechanics and exhibit high teno- and immuno-inductive potential on AECs, making them a potential candidate to be applied for surgical purposes in tendon regeneration.

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Mohammad El Khatib and Valentina Russo contributed equally to this work.

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keywords: Tendon Tissue Engineering, Electrospun 3D biomimetic scaffold, Tenodifferentiation, Immunomodulation, Amniotic Epithelial Stem Cells.

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A SURFACE TREATMENT OF NITINOL IMPLANTS REDUCES BLOOD ACTIVATION BY ALTERED PROTEIN ADSORPTION

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Introduction
Due to their favorable elastic properties, corrosion resistance and biocompatibility blood-contacting Nitinol is the material of choice for devices to treat cardiovascular diseases. A major drawback however is their strong thrombogenicity, making the use of systemic anticoagulation inevitable. Therefore, there is an urgent clinical need for reducing the thrombogenicity of Nitinol. We describe a simple surface treatment including the removal of surface contaminations and functionalizing the surface with phosphate ions. This treatment rendered commercially available Nitinol highly hydrophilic and anti-thrombotic. We investigate here the efficacy and mechanism of this treatment by comparing standard and surface treated Nitinol samples in terms of blood contact activation, cell adhesion, protein adsorption and endothelialization.

Methods
Nitinol discs and braids were tested. Discs were electropolished and passivated whereas the braids were thermally oxidized after electropolishing. These Standard (S) samples were washed with 0.9% NaCl prior to usage. Treated (T) samples were additionally treated with oxygen plasma, functionalized with KH_2PO_4, sealed with trehalose and washed with Hanks prior to usage.

The surface chemistry was analyzed by X-ray photoelectron spectroscopy (XPS) and its wettability by water contact angle measurements.
To investigate blood activation and clot formation, T and S braids were incubated statically and dynamically for 1h in fresh, minimally heparinized whole human blood. Blood activation was quantified by measuring thrombin-antithrombin complex (TAT) and β-Thromboglobulin (β-TG) concentrations in the blood plasma. Adherent human blood components were visualized by immunofluorescence and by scanning electron microscopy.
Blood plasma proteins adsorbed on S and T disks were analyzed by a standard proteomic work flow.

To study the mechanism of blood activation, S and T braids were pre-coated with 50 nM and 200 nM active FX (FXa) and inactive FX for 1 h in Hanks prior to static blood incubation tests. In whole human blood FX and FXII were inhibited by addition of Rivaroxaban (1-25 µg/ml) and FXII900 (1-100µM), respectively.

Endothelialization on S and T disks was evaluated by observing cell coverage of GFP-HUVECs over 4 days and quantifying the fluorescence signal.

Results
T surfaces show significantly lower water contact angles (≤ 10°) than S surfaces (70°-90°). XPS measurements revealed increased amounts of phosphate, calcium, and magnesium ions on T. Static and dynamic blood incubation tests showed a drastic reduction of thrombus formation, decreased TAT and β-TG concentrations, and reduced fibrin fiber deposition on T devices. Proteomic analysis of adsorbed proteins showed reduced protein abundance on T compared to S surfaces, including proteins of the complement pathway. Interestingly, the treatment increased the adsorption of calcium-binding vitamin K-dependent proteins including FX. Static blood incubation tests with FXa precoated braids resulted in blood activation on T surfaces while Rivaroxaban and FXII900 inhibited blood activation on S surfaces. The endothelialization was not altered upon surface treatment.

Conclusions
The herein presented treatment made Nitinol surface ultra-hydrophilic and anti-thrombotic. We propose that the strong hydrophilicity and the presence of phosphate and calcium ions steer the adsorption, conformation, and activation of blood proteins. We propose that this surface treatment might be applicable to all titanium alloys.

keywords: Cardiovascular devices, Antithrombogenic treatment, Hemocompatibility, Intrinsic coagulation pathway

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DEVELOPING A 3D IN VITRO MODEL OF RECTUS SHEATH HEALING TO TEST HERNIA MESHES.

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Introduction
Fibroblast populated collagen matrices (FPCMs) are well established as a model for assessing the behaviour of fibroblasts. When used on a fixed substrate, FPCMs have also proven to be a reliable model for the study of tissue healing. Until now, such techniques have only been used with dermal fibroblasts to examine skin healing. Our work will develop a fixed (tethered) FPCM seeded with rectus sheath fibroblasts that will act as an in vitro model of rectus sheath healing. We will use this model to test the effects of hernia mesh upon fascial healing in vitro. We hope this will mark the earliest iteration of an evolving model of rectus sheath healing that may standardize the process of pre-clinical biomaterial testing.

Methods
FPCMs will be formed using rat-tail collagen seeded with fibroblasts taken from human rectus sheath. FPCMs will be formed in custom 3D-printed rectangular moulds. Such moulds will fix the collagen gel at each far end - establishing a uniaxial tension across the gel. This tension will mimic that which lies perpendicular to a fascial wound closure –representing an in vitro model of early wound healing/ contraction and fascial granulation. During culture, 5 different polypropylene hernia meshes will be suspended mid-way through the collagen gel so that they may be incorporated into the tissue model. After 3-5 days, samples will be plastic compressed – leaving a dense collagen structure analogous to human rectus sheath. Each mesh will be tested at two perpendicular orientations for different culture lengths. Once formed, collagen/ mesh constructs will be assessed for their collagen alignment and their tensile strength.

Results
Thus far, our custom moulds have established a reliable ability to form contracting FPCMS which stimulate fibroblast and collagen alignment when formed at 1.5x106 cells/ml. We are able to successfully incorporate different hernia meshes into our model and test them through fluorescence microscopy and uniaxial tensile strength testing.

Conclusion
This new methodology shows promising early results. In the long term such tethered FPCMs may help develop a new generation of in vitro models for the testing of medical biomaterials

We present an ongoing development of a new technique for in vitro testing of hernia meshes, and their effects upon rectus sheath fibroblasts.

keywords: hernia, mesh, in vitro, testing

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EXPLORING THE BIOMATERIAL-INDUCED SECRETOME: PHYSICAL BONE SUBSTITUTE CHARACTERISTICS INFLUENCE THE CYTOKINE EXPRESSION OF MACROPHAGES

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In addition to their chemical composition various physical properties of synthetic bone substitute materials have been shown to influence their regenerative potential and to influence the expression of cytokines produced by monocytes, the key cell-type responsible for tissue reaction to biomaterials in vivo. In the present study both the regenerative potential and the inflammatory response to five bone substitute materials all based on β-tricalcium phosphate (β-TCP), but which differed in their physical characteristics (i.e., granule size, granule shape and porosity) were analyzed for their effects on monocyte cytokine expression. To determine the effects of the physical characteristics of the different materials, the proliferation of primary human osteoblasts growing on the materials was analyzed. To determine the immunogenic effects of the different materials on human peripheral blood monocytes, cells cultured on the materials were evaluated for the expression of 14 pro- and anti-inflammatory cytokines, i.e., IL-6, IL-10, IL-1β, VEGF, RANTES, IL-12p40, I-CAM, IL-4, V-CAM, TNF-α, GM-CSF, MIP-1α, Il-8 and MCP-1 using a Bio-Plex® Multiplex System. The granular shape of bone substitutes showed a significant influence on the osteoblast proliferation. Moreover, smaller pore sizes, round granular shape and larger granule size increased the expression of GM-CSF, RANTES, IL-10 and IL-12 by monocytes, while polygonal shape and the larger pore sizes increased the expression of V-CAM. The physical characteristics of a bone biomaterial can influence the proliferation rate of osteoblasts and has an influence on the cytokine gene expression of monocytes in vitro. These results indicate that the physical structure of a biomaterial has a significant effect of how cells interact with the material. Thus, specific characteristics of a material may strongly affect the regenerative potential in vivo.

keywords: β-tricalcium phosphate (β-TCP); cytokines; inflammation; macrophages; osteoblasts; peripheral blood monocytes; bone substitute materials

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EXTRACELLULAR PROTEIN IDENTIFICATION CYTOMETRY (EPIC) SINGLE CELL ANALYSIS

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Introduction
One of the main methods to investigate cellular function, including stem cell differentiation, pathological state, and drug responsiveness, is by analysing deposition of extracellular matrix (ECM). Current methods such as Western blot, mass spectroscopy, or immunostaining, either require the removal of cells and processing of the ECM prior to analysis, or only allow for the analysis of the ECM of a few hundred cells or less. In the first case, the ECM is destructively harvested and cellular heterogeneity is masked, while in the latter case too few cells are analysed to be able to draw conclusions regarding subpopulations within the cell population. Moreover, these techniques can only be employed to a limited extent to analyse ECM deposition in 3D cell culture. Hence, we propose Extracellular Protein Identification Cytometry (EPIC) as a novel method that allows for the quantification of specific pericellular matrix proteins of individual cells within a 3D microenvironment in an ultra-high-throughput manner.

Methodology
Human mesenchymal stem cells or chondrocytes were encapsulated in single cell microgels using droplet microfluidics. Standard photolithography and replica moulding was used to create patterned polydimethylsiloxane chips, which were bonded to microscope glass slides. Two different chip designs were used for droplet generation and delayed gelation respectively1. For single cell encapsulation1, detached cells (107 cells/ml) were resuspended in 5% Dex-TA dissolved in PBS mixed with 44 U/ml HRP and 8% OptiPrep. Flow rates were 6 µl/min, 2 µl/min, and 30 µl/min for the 2% Pico-Surf oil phase, aqueous phase with cells, and H2O2 solution respectively. Encapsulated cells were washed and subsequently transferred to chondrogenic medium and cultured for three weeks. For immunostaining, microgels were fixed using 4% PFA, permeated using TritonX-100, and subsequently blocked using 1% BSA for 1 h. Microgels were incubated with primary antibodies at 4°C overnight, washed with PBS, and incubated with secondary antibodies. DAPI was used as counterstaining. Imaging was performed with a confocal microscope. Quantitative fluorescence cytometry was performed with a FACS Aria II.

Results
Single cells were centred inside micrometre-thin microgels that could be cultured for three weeks with minimal cell escape1 allowing for matrix deposition. As a proof of concept, immunostaining for chondrogenic differentiation markers (e.g. COL II, ACAN, and COL I) was performed. Confocal analysis allowed for visualisation of targeted ECM protein(s) using indirect immunofluorescence. The fluorescent signal could also be detected with a FACS instrument, and flow cytometry analysis of a single antibody staining was performed.

Conclusions
Antibody staining of ECM proteins inside the hydrogel is possible and can be visualised. Also, it was shown that it is possible to detect and quantitate encapsulated cells using FACS, based on the immunostaining of a pericellular matrix protein of interest. As a next step, quantitative multiplexed FACS analysis of several deposited pericellular matrix proteins is going to be investigated.

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keywords: extracellular matrix, flow cytometry, immunofluorescence, single cell analysis

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GRAPHENE OXIDE NANOPLATFORMS TO ENHANCE PT-BASED DRUG DELIVERY IN OSTEOSARCOMA ANTICANCER THERAPY

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Introduction
Osteosarcoma is the most common type of bone cancer diagnosed especially in children and young adults1. A combination of chemotherapy, radiotherapy and surgery is commonly used to treat this type of cancer2,3. In detail, chemotherapy is based on the use of molecules targeting the high cancer cell proliferation metabolism such as Platinum-based drugs that binds nuclear DNA upon overpassing the cell membrane, causing its damage and the arrest of the cancer cell cycle at G2/M transition phase, leading to apoptosis4-7. Despite Pt chemotherapeutics are the most potent used anticancer drugs, their side effects (high degradation before entering the cells, the off-target organs toxicity, and cell resistance) remain great drawbacks8-11.

Materials and methods
In this study, we synthetized Graphene oxide (GO)-based nanoplatforms as smart delivery systems of Platinum-based drug. In order to reduce GO cytotoxicity in health cells while promoting its cellular uptake in cancer cells, and to allow Pt loading on GO, 8-arm polyethylene glycol-amine (PEG) was used. The bioactivity of GO-PEG-Pt platforms were compared to Pt-free (15μM, 30μM, and 60μM) on three osteosarcoma cell lines (MG63, U2 and SAOS-2). The in vitro analysis of cellular uptake (ICP-OES), viability (MTT assay), morphology (actin and DAPI staining) and migration (scratch test) was performed.

Results
A preliminary study showed that GO-PEG was not toxic for cells at any concentration tested compared to cells only. A significant cell viability reduction was detected at 30 μM GO-PEG-Pt for all cell lines compared to Pt-free, reaching 70% cell mortality in MG63 (p value ≤ 0.0001) and SAOS-2 (p value ≤ 0.001). Morphological analyses showed a round-shape cell morphology and cell number reduction in the presence of GO-PEG-Pt respect to Pt-free in a dose dependent trend. Cellular uptake of GO-PEG-Pt was significantly higher after 24h for SAOS (p value ≤ 0.05) and MG63 (p value ≤ 0.0001) cell lines than Pt-free. The cell migration was lower in Go-PEG-Pt than Pt-free in MG63 and U2 with overall more than 60% migration inhibition over time at 30 μM concentration.

Conclusions
The results confirmed that GO-PEG-Pt platforms work as promising anticancer delivery systems. In fact, all the three osteosarcoma cell lines showed higher susceptibility to GO-PEG-Pt in terms of lower metabolic activity and lower migration rates due to the higher GO-PEG-Pt uptake compared to Pt-free.

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keywords: Nanomedicine, Osteosarcoma, smart delivery nanosystems, Graphene Oxide, anticancer drugs

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IN VITRO ASSESSMENT OF THE DEGRADATION INTERFACE OF PURE MG BY DIRECT OSTEOBLAST AND OSTEOCLAST MONOCULTURE AND COCULTURE

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INTRODUCTION: Osteoblasts (OBs) and osteoclasts (OCs) interaction have an important role in bone remodeling [1]. Thus, coculture with both bone cells (OB-OC) is needed to achieve a better understanding of the material-cell interaction and the impact on Mg degradation for its potential use as a biomedical material. The aim of this study was to analyze the surface morphology and dynamic changes in the composition of the corrosion layer formed on Mg influenced by direct monoculture and coculture models.

METHODS: High purity Mg (99.94%) disc-shaped samples (diameter 9 mm, thickness 1.5 mm) were used in this study. The specimens were grinded, polished, and finally sterilized by gamma irradiation at a dose of 33.5 kGy.

α-MEM (Minimum Essential Medium) with 10% FBS (Fetal Bovine Serum) and 1% P/S (Penicillin/Streptomycin) was used as cell culture and immersion medium. Human primary OBs and OCs were pre-differentiated separately for 21 days (from HUCPV* and PBMC **, respectively) and seeded on materials as described in Ref [2] in the monoculture, and coculture model (OB 1: 2 OC ratio). Cells also were seeded on glass (herein, employed as controls). Changes in adhesion were evaluated by actin cytoskeleton staining after 7 and 14 days. Surface topography and cell morphology were analyzed on samples prepared by Critical Point Drying (CPD) by SEM (SU-8000, Hitachi). Cross-sectioning, imaging, and evaluation of distributed elements composing the degradation interface were analyzed by FIB/SEM/EDX (NB-5000 dual-beam, Hitachi) from samples prepared by CPD.

RESULTS: Cell spreading, the proliferation of differentiated OBs, and cell attachment of OCs were observed after 7- and 14-days cell culture on Mg. OBs extensively grew as elongated cells whereas rounded adhered multinucleated OCs showed their typical dorsal villus-like processes and adhesion to the Mg surface. After 14 days, the corrosion layer formed under OCs had slightly higher and uniform distribution weight percent (wt.%) oxygen content. Similarly, Phosphorus (P) and Calcium (Ca) were slightly higher in wt.% content and homogeneously distributed in the corrosion layer formed beneath the OB-OC coculture.

CONCLUSIONS: The results showed the successful cell attachment and the vitality of differentiated OBs and OCs on Mg surface in direct cell monoculture and coculture models after 14 days. A slightly distinct corrosion product was pointed out beneath OB-OC coculture with slightly homogenously increased wt.% of P and Ca compared to monoculture models after 14 days.

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* HUCPV: Human Umbilical Cord Perivascular Cells
** PBMC: Peripheral Blood Mononucleated Cells

keywords: Magnesium, Osteoblast, Osteoclast, Coculture, Degradation interface, Biomaterial

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INVESTIGATION OF FOREIGN BODY RESPONSE AGAINST SUBCUTANEOUS DIABETES-REVERSING IMPLANTATION BY UTILIZING RAMAN MICROSPECTROSCOPY

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Type 1 diabetes (T1D) is an autoimmune disease that leads to the destruction of pancreatic islets, resulting in the deficiency of insulin secretion. The current treatment is a daily insulin injection regimen, which negatively affects the quality of life for T1D patients and is difficult to manage over an entire lifespan. Therefore, an alternative treatment – islet transplantation within implantable devices – has been regarded as a promising therapy to achieve insulin-independence. Nevertheless, the interplay of implant surface and recipient tissue can result in a foreign body response (FBR) which is one of the main reasons for implant failure. The encapsulation isolates the implant from surrounding tissues, impeding it from nutrients, oxygen and drug molecule diffusion. In this study, a non-invasive method, Raman microspectroscopy (RM), was utilized to investigate the characterization of the extracellular matrix (ECM) within the fibrotic capsule, which can be utilized for future application in medical device assessment.

In this study, implantable devices were analyzed in regard to their potential to induce the FBR. A marker-independent approach via RM was applied to investigate the fibrotic capsule. True Component Analysis (TCA) was utilized to generate intensity distribution images for ECM components. ECM proteins were identified and validated by immunofluorescence (IF) staining. Principal component analysis (PCA) was conducted to assess further molecular information.

Fibrotic capsule structures could be identified by RM as well as IF and histological staining. Raman imaging and TCA allowed the identification, localization and quantification of collagen I, collagen III as well as α-SMA. PCA of extracted collagen I spectra indicated differences between collagen structures of fibrotic capsule and connective tissue, represented by shifts in Raman bands assigned to amide I and III, phenylalanine and hydroxyproline. This study demonstrated that RM has the potential to examine the severity of the FBR.

keywords: Foreign body response, Extracellular matrix, Raman microspectroscopy

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MAGNESIUM IMPLANT DEGRADATION FOR BONE TISSUE REGENERATION – CIRCULATING BIOMARKERS OF INFLAMMATION AND BONE REGENERATION IN A RODENT MODEL

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Introduction: Biomechanical features and osteogenic properties of magnesium implants make them suitable for orthopedic applications. [1] Commonly used in vivo methods to investigate tissue response, such as histology and gene expression of peri-implant tissue, are terminal procedures that give a limited temporal and spacial view on the organism response to the implant. [2] Thus, systemic biomarkers of bone turnover currently used to diagnose and monitor musculoskeletal diseases may be of great value in the context of magnesium degradable implants. Biomarkers profile characterization can be used to follow the successful integration of the implant and tissue healing as well as addressing more accurate implant designs and chemical composition. In this study, we analyze systemic inflammatory and bone healing markers pattern expression after magnesium implantation and aim to correlate it with results of histopathology and implant surface analysis.

Methods: Animal experiments experiments are approved by Instituto Superiore di Sanità on behalf of Italian Ministry of Health and Ethical Panel (Prot. n° 299/2020-PR) and local ethics committee. Female Wistar rats (n=28) 12-week old were used to press-fit WE43 pins (1.6mm diameter *8mm length) transcortically into the diaphyseal region of the femur of both legs. Before surgery, and 1, 3, 7, 14, 28, 45 and 90 days after surgery, blood samples were collected for the analysis of inflammatory (IFNγ,IL-10 IL-1 , IL-4, IL-6, CXCL1, MCP-1, TNF ,VEGF) and bone regeneration (Osteopontin, Osteoprotegerin, FGF-23, DKK1, TIMP-1)markers using LUMINEX Immunoassay. Furthermore, on days 3,7,14 and 90 some animals were euthanized, one leg was used for histological H&E staining and the other for pins retrieval and SEM surface analysis

Results: Systemic markers such as VEGF(vascular endothelial growth factor) and OPN (Osteopontin), an early marker bone regeneration, present a peak 3 days after surgery followed by a decrease. At day 45, OPN show significant increase possibly associated with a more advanced stage of bone remodeling. The ex vivo surface analysis in the retrieval implants showed that since day 3 there was a formation of a corrosion layer with a cracked appearance. EDX results revealed that Oxygen (O), Magnesium (Mg), Phosporus (P), Calcium (Ca), and traces of alloying elements were the elements composing the corrosion layer. Moreover, the weight percent content (wt. %) of O, P and Ca increased gradually with time.

Conclusion: Our preliminary biomarkers results show a time course evolution of specific biomarkers and the pin surface analysis of explanted pins also show an increased content of O, P, Ca (wt.%) with implantation time. The use of biomarkers to assess the impact of degradable implants on the bone and whole organism is a promising starting point as a monitoring tool to

follow the evolution of implant degradation and tissue regeneration.

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keywords: magnesium, degradation interface, inflammation, biomarkers, bone regeneration

20941834888

MECHANO-INDUCED CHONDROGENESIS OF HUMAN MSCS IN A BIOMATERIAL: A FACTORIAL DESIGN OF EXPERIMENT APPROACH

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Introduction:
The in vitro differentiation of bone marrow-derived mesenchymal stem/stromal cells (BM-MSCs) to chondrocytes is mainly driven by the exogeneous administration of transforming growth factor β (TGF-β)[1]. Multiaxial load-induced activation of TGF-β1 (TGF-β1), which is secreted by the cells in a latent form has been shown to drive chondrogenesis[2]. In the present work, we apply a factorial design of experiment (DOE) and planned contrasts to establish the main and interactive effects between different loading parameters/factors in order to find a loading protocol that maximizes TGF-β1 activation in a biomaterial. Furthermore, we investigate whether the different loading protocols lead to changes in the secretion of biomarkers that affect chondrogenesis, such as bone morphogenetic protein 2 (BMP2).

Methodology:
BM-MSCs were obtained from three donors after acquiring written consent from the patients (Freiburg, EK-326/08) and encapsulated in fibrin–poly(ester-urethane) scaffolds (5 x 106 cells per scaffold). Samples were cultured in TGF-β1-free chondropermissive medium either in an unloaded state or subjected to joint-mimicking multiaxial load (combination of shear and compression) within a multiaxial bioreactor. The loading protocol consisted of 1 h of loading per day during a period of 12 days. Different combinations of three factors, namely type of indenter, shear frequency and compressive strain were used to investigate the effect of the factors' interaction on biomarker secretion. Two types of counterfaces were used: a cylinder and a ball. Two different settings were used for the shear frequency (0.2 and 1 Hz) and for the compressive strain (5% and 20%). The setting at 0.6 Hz shear frequency and 10% compressive strain represents the center point that demonstrates whether the relationship between factors is linear and to ensure process stability. Culture medium was collected and replaced every 2/3 days. ELISAs were performed to quantify BMP2 and TGF-β1. Nitrite was detected using the Griess assay and sulphated glycosaminoglycans using DMMB. The different outputs were all normalized to the DNA content (Hoechst dye).

Results:
Among the different donors, both active TGF-β1 and BMP2 showed very similar trends (r = 0.88). A protocol could be selected that resulted in the highest values for both biomarkers. Significant interaction could be found between the different factors using contrast analysis and a linear mixed model. DNA content remained consistent across all groups.

Conclusion:
We show that varying multiple parameters does have an effect on biomarker secretion. Active TGF-β1 and BMP2 were highest with a very specific loading regime (cylinder, shear frequency: 1 Hz, compressive strain: 20%). Certain biomarkers seem to be differentially regulated by mechanical stress. We conclude that a DOE is a useful tool to quickly screen complex relationships between multiple different factors in a biomaterial, which would take much longer in a one-factor-at-a-time approach.

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keywords: DOE, contrasts, Bioreactor, biomaterial, TGF-β1

73296313324

NEURONAL DIFFERENTIATION BY DYNAMIC PIEZOELECTRIC STIMULATION

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Introduction: Electroactive smart materials are increasingly being used for tissue regenerative applications. Piezoelectric polymers, such as poly(vinylidene fluoride) (PVDF), are one type of electroactive materials that generate electrical potential fluctuations simply by applying mechanical pressure to the material, without the need for additional power sources. The use of piezoelectric PVDF as an electroactive material to enhance neural differentiation has been examined in this study.
Methodology: Human neural precursor cells (hNPCs) were cultured on piezoelectric poled and non-poled β-PVDF films with or without a pre-coating step of poly-D-lysine and laminin (PDL/L), and differentiation into the neuronal lineage was assessed (MAP2+ and DCX+) under static or dynamic (piezoelectric stimulation) culture conditions.
Results: The results revealed that under static culture conditions, PVDF films promote neuronal differentiation, which is amplified when the films are mechanically stimulated. Furthermore, in silico studies of the electrostatic potential of different domains of laminin (L) revealed that they are mostly polar, implying that highly polar PVDF films interact more with the fluctuating surface electric field under mechanical stimulation.
Conclusions:These findings support the higher neuronal differentiation induced by poled β-PVDF films pre-coated with PDL/L under dynamic conditions. Overall, results demonstrate that electromechanical stimuli created by piezoelectric PVDF films are suitable for promoting neuronal differentiation and should be investigated for neuroregenerative therapeutic approaches.

keywords: piezoelectric materials, PVDF, human neural precursor cells, neuronal differentiation

83767206328

PARAMETERS DRIVING THE FIBROTIC ENCAPSULATION OF IMPLANTABLE HYALURONAN-BASED MATERIALS

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Introduction
Implantable materials, e.g. barriers hindering the formation of peritoneal adhesions, are often produced from natural biopolymers. It is generally assumed that using biocompatible polymers such as hyaluronan (HA) will ensure the safety of the resulting product. But even products based on HA can elicit foreign body response (FBR) leading to their encapsulation (and thus to lower efficiency of treatment). The first reaction to the implanted material is protein adsorption. The protein layer can then facilitate cell adhesion. In this work, we studied the ability of different HA-based implantable films to attract proteins and cells both in vitro and in vivo in a mouse model. We correlated the results with the level of FBR induced by these films after implantation in the mouse peritoneum.

Methodology
Two commercial HA-based films (Seprafilm and Hyalosafe) and several in-house prepared films from either hydrophobized HA or cross-linkable HA derivatives were tested. The films were characterized by water contact angle and swelling degree measurements. Their solubilization and HA release rates were determined by LC-MS. These materials were implanted in the peritoneum of healthy mice and collected after 15 min or incubated for 15 min in vitro with different protein solutions including FBS and partially heparinized blood. These materials were then used for proteomic analysis of adsorbed proteins and in vitro cell adhesion analysis.

Results
Standard model biological fluids, such as FBS or blood, yielded different protein spectrum adsorbed onto materials than the real peritoneal fluid in vivo. We consequently improved the in vitro protocol to better reflect the in vivo conditions: films were first treated with a solution of fibrinogen and thrombin. This increased their cell adhesiveness to the level observed in samples incubated in the peritoneum. However, the film's ability to adsorb proteins was found to be of secondary importance. Instead, film stability in biological media measured by the release of HA and evidenced by material gelation or fragmentation proved to be decisive for cell adhesion. Film stability correlated with the observed frequency of FBR.

Conclusions
The often-used biological media such as FBS, blood, or BSA solution are not good models for peritoneal fluid and possibly other body fluids due to the limited spectrum of protein they contain. Hyalosafe is made of hydrophobized HA (with 100% degree of substitution), but surprisingly its surface is hydrophilic. It is also the most stable biomaterial tested and is known to elicit FBR. In-house materials both from partially hydrophobized HA and hydrophilic

crosslinked HA were able to support the adhesion and growth of cells only in the more stable variants. Those solubilized after 1-3 day in culture media was generally non-adhesive for cells after implantation similar to Seprafilm. Thus, the biocompatibility of the film material itself or its properties such as hydrophilicity were not sufficient to prevent the FBR.

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keywords: peritoneum, hyaluronan, film, cell adhesion

62825423646

PLATINUM CONJUGATED TO NOVEL GRAPHENE OXIDE NANOPLATFORMS AS ANTICANCER THERAPY FOR GLIOBLASTOMA AND BREAST CANCER

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Introduction
Glioblastoma is a very aggressive type of cancer with a very poor life expectancy for patients and breast cancer often metastasizes into the liver, lungs, brain, and, in 70% of cases, to bones[1,2]. Chemotherapy is largely used to treat cancer and it is based on the use of molecules targeting the high cancer cell proliferation metabolism[3]. Platinum (Pt) and three of its isoforms (cisplatin, carboplatin, and oxaliplatin) are some of the most successful metal-based drugs to cure breast cancer and glioblastoma[4,5]. Despite Pt-based chemotherapeutics being effective, their side effects (high degradation before entering the cells, the off-target organs toxicity, and cell resistance) remain great drawbacks[6-9]. In this work, it was developed a Graphene Oxide (GO) nanoplatform functionalized with Pt as a promising smart delivery system that could increase the Pt cellular uptake reducing the Pt amount needed for cancer treatment and consequently the side effects.

Materials and methods
GO nanoplatforms were treated with 8-arm polyethylene glycol-amine (PEG) that permits to load Pt on the platform (GO-PEG-Pt) and an extensive in vitro screening was performed on two breast cancer cell lines with aggressive nature that lead to metastatic behavior (MDA-MB 231 and MDA-MB 468) and two glioblastoma cell lines (U87 and U118). The bioactivity of GO-PEG-Pt compared to Pt-free (15μM, 30μM, and 60μM) was analyzed looking at the effect on cellular uptake (ICP-OES), viability (MTT Assay), morphology (DAPI and actin staining), and migration up to 72 hours (Scratch Assay).

Results
The cell viability was significantly lower in MDA-MB 468 and U118 cells at 30μM for GO-PEG-Pt group compared to Pt-free (<75%), and even the cell morphology seemed to be compromised. These results were highly related to the cellular uptake of GO-PEG-Pt which is significantly higher compared to Pt-free after 24h. This data confirmed that our nanoplatform promotes drug delivery directly inside the cells. In addition, GO-PEG-Pt mostly affected the cell migration compared to Pt-free, in particular, MDA-MB 231 showed a migration reduction of 60%, and this could be a great advantage in reducing the metastasis process.

Conclusions
This study demonstrated that the combination of Pt onto PEG-functionalized nano-sized GO provided numerous advantages for tumor therapy such as minimizing toxicity, enhancing the cellular uptake, and consequently we could reduce the side effects because a lower amount of Pt is necessary.

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keywords: delivery system, breast cancer, glioblastoma, graphene oxide, nanoplatforms

20941828124

RNASEQ ANALYSIS REVEALS DIVERGENT MOLECULAR EVENTS THAT DIRECT HBMSCS TOWARD FIBROSIS OR BONE REGENERATION: IMPORTANCE OF INFLAMMATION REGULATION

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Human bone marrow mesenchymal stromal cells (hBMSC) combined with biomaterials are currently used clinically for bone repair, but their bone formation kinetics are heterogeneous. The development of optimized pro-regenerative therapies requires a rigorous understanding of the molecular progression of regeneration. Here we report the divergent molecular events that drive hBMSCs toward fibrosis or bone regeneration. To understand this heterogeneity, we focused on the behavior of hBMSCs and observed that hBMSCs with high bone potential were associated with higher cell survival and proliferation after in vivo transplantation. In order to determine their mechanism of action and to understand how cell survival is controlled, RNAseq analysis was performed at 8h, 1 and 2 weeks after transplantation. Our results show that cells with reduced bone potential have a higher expression level of pro-inflammatory cytokines at 8 hours post-graft, which is associated with a higher inflammatory response. We then confirm that the long-term survival of hBMSCs depends in part on their ability to regulate neutrophil activation. Furthermore, our RNAseq analysis at 1 and 2 weeks highlights that long-term survival of hBMSCs is associated with increased bone formation only if hBMSCs are able to evolve towards osteoblastic differentiation. Our hypothesis is that bone differentiation allows hBMSCs to synthesize paracrine factors necessary for chemoattraction and osteoblastic and osteoclastic differentiation of neighboring cells. However, the heterogeneity of the cells' potential is partly due to their ability to regulate the inflammatory response. Our results will serve as an omics map of hBMSCs for bone regeneration and may have therapeutic implications for managing the inflammatory response.

keywords: hBMSC, RNAseq, bone regeneration, inflammation, neutrophil

94238165597

SELECTED TECHNOLOGICAL AND BIOLOGICAL FACTORS DECIDING ABOUT SCAFFOLD FUNCTIONALITY

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The quality of life of millions of patients has been improved by the development materials for regenerative medicine such as total knee joint prostheses, cardiovascular stents, orthopaedic implants, as well scaffolds for tissues regenerative. The global regenerative medicine market size is expected to reach USD 57.08 billion by 2027, growing at a CAGR of 11.27% over the forecast period.
In general, materials for regenerative medicine needs to be biocompatible, nontoxic, and fulfil properties suitable for specific application. Additionally, scaffold needs to fulfil different chemical and mechanical requirements due to differences in microenvironment of the impanation..
The aim of this presentation is to show selected research done in topic of crucial factors effecting scaffold functionality.
Fundamental research investigations about electrospun scaffolds development, structural and surface properties in terms of material-cells interaction will be analysed. We proved, electrospinning parameters and crystallinity degree play important role in cellular- material interaction.
Our second field of interest are grafts for ligaments reconstruction. Analyses of electrospun materials modified with growth factors were perform and effect of this modification on selected on gene expression of mesenchymal stem cells was analysed.
Finally I will present preliminary data from our last project “Preclinical study of the implant for reconstruction of the cruciate ligament with a substitute for bone regeneration”, acronym BioLigaMed (Small Grant NOR/SGS/BioLigaMed/0272/2020).

keywords: polymers, scaffolds, tissue engineering, crystallinity, growth factors

94238123705

TAILORING GELAGE-BASED HYDROGELS TO SUPPORT LONG-TERM SURVIVAL AND FUNCTION OF PRIMARY HUMAN CELLS

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Introduction: Living tissue is a very complex biological construct made of cells, signaling molecules and multiple extracellular matrix (ECM) components. Each of these elements is equally important to keep the homeostatic balance of tissue and to control the functions of organs. This heterogeneity of living tissues makes their engineering in laboratory conditions challenging 1. Biocompatible hydrogel systems are one of the favored ECM replacement materials, since they provide porous microenvironment with the possibility of nutrient and oxygen exchange. However, it is not possible to employ one hydrogel system for all cell types. Different cell types require specific stiffness, viscoelasticity, or hydrogel chemistry to survive. In this study, we optimize an allyl-modified gelatin (gelAGE) hydrogel to recreate an ideal microenvironment which supports long-term survival and functionality of fibroblast and endothelial cells.

Methodology: GelAGE and multi-arms thiolated polyetylenglycol are photo-crosslinked using thiol-ene click chemistry and characterized using rheology and photo-rheology analysis, as well as swelling and static compression tests. Primary human umbilical vein endothelial cells (ECs) and normal human dermal fibroblasts were casted within the hydrogel precursor solution individually or as co-culture and crosslinking of hydrogels was performed under UVA light. Cell viability and proliferation was determined by live/dead staining and fluorometric measurement of dsDNA content. Cell morphology was visualized with Hoechst and Phalloidin staining. Soluble fibronectin and soluble collagen from all samples were measured by fluorometric methods with respective assay kits. Expression of CD31 and fibronectin were confirmed by immunofluorescent staining. Furthermore, cell spheroids embedded in hydrogel were monitored in real-time using IncuCyte spheroid module, to evaluate the migration of cells out of spheroids through the hydrogel.

Results and conclusion: Two different GelAGE types (G1MM and G2LH), adapted from literature 2, have shown different molecular properties (molecular weight and degree of modification) and physico-chemical properties. G2LH shows longer polymer chains and less substituent moieties. Cell viability and morphology of ECs and fibroblasts were compared between GelAGE1MM and GelAGEG2LH. Both hydrogel types supported cell viability, but cells embedded in GelAGE1MM remained rounded and did not show any morphological alterations until day 28. In contrast, fibroblasts embedded in GelAGEG2LH begun to elongate on the first incubation day, and ECs on day 3. Further biological analyses were therefore performed only with GelAGEG2LH-embedded

cells. Biological activity of individual cell types was supported in GelAGEG2LH hydrogels. Time-dependent secretion of soluble collagen and fibronectin was detected in both cell types, but was, expectedly, dramatically higher in fibroblasts than in ECs. Immunocytochemical analysis of co-cultures indicated that endothelial cells expressed CD31 and formed cell-cell contacts. Furthermore, strong fibronectin expression in all cells, as well as vessel-like structural organization at day 14, were observed. Real-time monitoring of cell spheroids embedded in GelAGEG2LH hydrogels showed that cells started to move out of the spheroids into the hydrogel after 5 days of incubation. Taken together, our results demonstrated that GelAGEG2LH hydrogels support cell viability, biological function and motility, and may thus fulfill the demands of different cell types thanks to the respective chemical modifications.

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keywords: GelAGE, extracellular matrix, primary cells

PS50
One health, one medicine: What Veterinary regenerative medicine can teach us

20941844888

ANALYSIS OF HUMORAL RESPONSE TO EQUINE MESENCHYMAL STEM CELLS (MSCS)

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Introduction
In recent years, there has been growing evidence that mesenchymal stem cells (MSCs) are not truly immune-privileged but immune-evasive, and thus, their recognition and elimination by the immune system in the allogeneic setting should be considered. In this regard, antibody production after MSC allogeneic administration MSCs has been demonstrated in several species, including the horse, which is highly relevant both as patient and as translational model. The development of immune memory mechanisms could impact MSC clinical application and potentially limit their repeated administration. Proinflammatory priming of MSCs can potentiate their regulatory ability in vivo but increased expression of the major histocompatibility complex (MHC) might also increase their immunogenicity. Moreover, MSC differentiation may influence both their immunomodulation and immunogenicity.
This study will assess the humoral immune response to equine MSCs, unmanipulated (MSC-naïve), pro-inflammatory stimulated (MSC-primed) and chondrogeneically differentiated (MSC-chondro) by evaluating the production of cytotoxic allo-antibodies directed against donor's equine leukocyte antigen (ELA) after autologous and allogeneic MHC-matched and mismatched administration.

Methodology
Three animals homozygous for specific MHC haplotypes were selected as MSC donors. Equine MSC-naïve, MSC-primed or MSC-chondro were obtained and encapsulated in alginate hydrogels, and subcutaneously placed in either the donor horses (autologous) or in MHC-matched or MHC-mismatched recipient horses. Each animal received three MSC implants with the same type of MSCs on one neck side. The implants were removed after 1, 3 and 6 weeks. One month after

removing the last implant, each animal was re-exposed to the same MSCs by repeating the whole procedure in the contralateral neck side to assess the possible development of immune memory mechanisms.
Peripheral blood from each horse was collected before implant placement and serially at each time-point of removal. The sera were harvested and used to assess the humoral immune response over time and under the different conditions by using microcytotoxicity assays, in which sera were exposed to MSCs from the corresponding donor. This methodology has been previously described to detect the presence of cytotoxic allo-antibodies after MSCs administration in the horse. MSC targeting was assessed using the scoring system previously described: score 1 < 10%; score 2, 10–19%; score 4, 20–49%; score 6, 50–80%; score 8, 81–100% of dead cells.

Results
According to previous results, autologous and allogeneic MHC-matched recipients did not develop antibodies against the ELA of the MSC donor, regardless of having received MSC-naïve, MSC-primed or MSC-chondro. All the samples of these animals were scored as 1 or 2, which reflects non-significant presence of allo-antibodies in sera.
However, higher scores were observed in sera from animals receiving MHC-mismatched MSCs in the tree conditions, reflecting recognition by the recipient’s immune system and the production of allo-antibodies.

Conclusion
The compatibility between donor and recipient is a key point to consider in order to avoiding the production of immune memory mechanisms against allogeneic MSCs, including antibody formation against donor’s MHC. The immune response elicited against equine allogeneic MSCs and the factors influencing it must be further studied to develop more effective and safer cell therapies.

keywords: mesenchymal stem cells, horse, immune response, microcitotoxicity, antibody

PS51
Perspectives and Challenges in Bioengineering Dynamic Hydrogels for Regenerative Medicine

52354519444

DETERMINATION OF MATERIAL PROPERTIES IN SOFT AND DENSE COLLAGEN TYPE I GELS USING OSCILLATORY RHEOMETRY

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INTRODUCTION
Biophysical parameters are integral to various pathophysiologies, such as fibrosis and cancer. Systematic investigations of such parameters and how they influence cell behaviour and disease progression are feasible in 3D in vitro models of disease.
In this project we used collagen I, the most abundant extracellular matrix protein, to engineer 3D constructs, both as soft hydrogels and dense models. Many techniques have been used to explore biomechanics but limitations surrounding methodology and different elastic moduli, makes direct comparison of measurements difficult.
We aimed to establish a reproducible technique to measure shear elastic modulus, as a measure of material stiffness, by oscillatory rheometry.

METHODS
3D Collagen I Scaffolds: Acellular soft and dense collagen type I gels, were manufactured with a starting concentration of 2 mg/ml. To fabricate hydrogels with 0.2% density a mix of collagen type I, 10xMEM and neutralising agent composed of 10 M NaOH was prepared and 1 M HEPES buffer. This mix was set for 15 mins at 37oC.
Dense scaffolds were created by compressing hydrogels using RAFT absorbers (RAFTTM) to expel liquid, for a final collagen density of 10%. All Scaffolds were maintained in RPMI 1640 with 10% FBS 1% P/S, in a humidified atmosphere at 37°C, 5% CO2 air.
Rheology: Amplitude sweep and frequency sweep testing was conducted on collagen type I scaffolds, using the Kinexus Pro+ Rheometer (Netzsch). 20 mm parallel plate geometry with a solvent trap was used, testing at 1Hz frequency and 37°C. Testing determines shear complex modulus (G*), which defines the elastic storage modulus (G') and viscous loss modulus (G''), and phase angle (θ). Testing parameters set by normal force (N) or gap size (h). Storage modulus (G'), phase angle (θ), LVE region and stress (σ) strain rate ( ) curves were analysed. Moduli were calculated by averaging the last 10 points in the LVE region.
RESULTS
Dense collagen scaffolds had a higher storage modulus, averaging between 578 Pa ±181 (S.D.). Whereas soft gels exhibited lower storage moduli around 33 Pa ± 4.7 (S.D.). Strain stress curves from amplitude sweep indicate a longer linear viscoelasticity region (LVER) in soft scaffolds, with phase angles between 7° and 9°. In comparison, dense gels had phase angles between 10° and 11°.
Frequency sweep testing indicates the collagen scaffolds behave as viscoelastic solids. Setting testing parameters by normal force or gap size showed greater stiffness in dense gels. However, overall storage moduli measurements were higher in all scaffolds when setting by normal force.

CONCLUSION
Collagen density influences the tissue's mechanical properties as soft scaffolds exhibited greater viscoelasticity compared to dense constructs. These scaffolds can mimic physiologically relevant

tissue stiffness, which can be used to investigate the interactions between key cell types and the biophysical microenvironment.
Testing by set normal force resulted in measurements being higher, although those experiments also had much lower gap size compared to experiments set by gap. The sensitivity of the construct's biomechanics in response to stress is evident. Further understanding of this will guide our design for more biomimetic 3D disease models.

ACKNOWLEDGEMENTS
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keywords: Rheology, ECM, Collagen, Scaffold, Mechanics

94238123804

DEVELOPING LOW COST AND NON-ANIMAL DERIVED DYNAMIC HYDROGELS FOR TISSUE ENGINEERING

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Biomedical research is in need of evolving towards more sustainable models by adopting the concept of the three Rs (reduction, refinement, replacement of animals in research). Additionally, economic limitations in developing countries should also be taken into consideration. This project aims to develop a non-animal sourced and low-cost biomaterial that can be used to create accurate tissue models. We have developed a novel alginate-derived hydrogel, cross-linked with oxime bonds. These dynamic covalent linkages lead to form mechanically robust and stable structures with self-healing properties. These properties mimic natural characteristics of some human extracellular matrices. Our long term goal is to further functionalise with bioactive ligands also using oxime bonds. The output of this work will provide a platform from which to develop in vitro tissue models, based on affordable and renewable starting materials, suitable for the expansion of biomedical research in developing countries.

keywords: Alginate, Biomaterial, Low cost, Dynamic Material

94238115967

HUMAN PBMC CONTRIBUTION ON MYOGENIC COMMITMENT OF HUMAN MESENCHYMAL STEM CELLS BY MYOBLAST 3D CO-CULTURE

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Symposium: Cell-rich constructs for tissue engineering.
Introduction: The development of in-vitro model of skeletal muscle regeneration is extremely challenging. Human Bone Marrow-derived Mesenchymal Stem Cell (hBM-MSCs) and human Myoblast co-culture with growth factor supplementation has been described for in vitro myogenic commitment [1-2]; among them, b-Fibroblast Growth Factor (b-FGF) was largely used at concentrations of 10 ng/mL [2]. On the other hand, human Peripheral blood mononuclear cells (hPBMCs) were also described as significantly involved in myogenesis [3]; however, their contribution is still under debate. Thus, to better understand their role, in our study, hPBMCs were added to an in vitro 3D co-culture of BM-MSCs and myoblasts (hSkMs) to investigate their potential contribution on hBM-MSC myogenic commitment.

Materials and methods: In vitro 3D scaffold-based systems were assembled using fibrin hydrogel cultured in perfusion conditions, and three cell types were seeded together. 2D in vitro culture of human BM-MSCs and hSkMs in the presence of PBMNCs placed in an upper chamber of transwell insert was adopted as control.

Results and Discussion: hBM-MSC commitment towards a myogenic phenotype was confirmed by qRT-PCR showing upregulation of myogenic-related genes, such as Pax3, MyoD1, Myf5, Myf6, Desmin and Myosin Heavy Chain II (MYH2). In particular, Desmin expression showed an increasing trend in the presence of PBMNCs, suggesting a synergist paracrine effect of peripheral blood cells on myogenic commitment. The presence of Desmin and MYH2 at protein level was also confirmed by immunofluorescence assay. Finally, the 3D environment in dynamic culture was an extremely favorable biomimetic environment allowing the study of all myogenic marker expression at both gene and protein levels.

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keywords: 3D scaffold, myogenic commitment, perfusion system

20941853487

SUPRAMOLECULAR MICROGELS AS TUNEABLE 3D CELLULAR MICROENVIRONMENT

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Introduction
Tissue engineering entails the combination of mechanical, chemical and biological components to process materials that can be engineered towards artificial tissue structures in order to mimic the natural microenvironment of cells; the extracellular matrix (ECM), which provides cells with structural support as well as biochemical, biophysical, and mechanical cues. Biomaterials have been extensively studied as two- or three-dimensional ECM-mimicking substrates for tissue engineering. Cells seeded on these materials indicate how material cues such as stiffness, porosity, and adhesion-ligands influence cellular mechanisms. However, these material cues are usually coupled to each other and effect the cell population as a whole. Additionally, neighbouring cells, soluble factors and biophysical fields also influence cellular behaviour. This complex, heterogeneous and dynamic cell microenvironment makes it challenging to identify the effect of individual material cues on individual cell behaviour.

Engineered cellular micro niches are promising to study cell–material interactions. This could be achieved by the encapsulation of single cells in a micro-hydrogel (microgel) with droplet microfluidics. For this purpose, synthetic microgels were generated based on supramolecular ureidopyrimidinone (UPy)-based molecules. Two of these molecules can self-assemble by the formation of non-covalent complementary quadruple hydrogen bonds.1 Bioactivity can be easily introduced to the hydrogel system by modular approach via the incorporation of UPy-functionalized additives. The modularity of the UPy-hydrogel system provides the opportunity to create a variety of different hydrogel compositions.

Methodology
In this research, supramolecular microgels with different weight percentages were generated using droplet microfluidics according to Sinha et al. (2019).2 Two types of building blocks were designed for the formulation of the microgels: the bifunctional UPy-PEG10K-UPy (BF) and the monofunctional UPy-glycinamide (MF). The BF and MF molecules were both dissolved in 80 mM NaOH at 70 °C 1 hour while stirring and after cooling down neutralized with 1 M HCl, whereafter the droplets were generated. Thereafter, the droplets were observed using confocal microscopy. In addition, induced pluripotent stem cells (iPSCs) were mixed in the BF solution and used with 3 mM of the cell adhesive UPy-cRGD integrin-binding peptide to generate cell encapsulated microgels.

Results
Picolitre-sized supramolecular microgels (diameter = 79.2 ± 6.5 µm, n = 9) were generated using droplet microfluidics. The use of different weight percentages (0.625, 1.25, and 2.25 wt%) result in microgels with different stiffnesses ranging from ~ 100 – 1000 Pa. The incorporation of 1 mM fluorescent UPy-Cyanine 5 additive in the microgels showed a homogeneous distribution of the dye throughout the whole microgel, which is promising for the incorporation of other additives. Ongoing research is conducted on the encapsulation of iPSCs in microgels.

Conclusion
UPy-based supramolecular microgels were successfully generated by droplet microfluidics. This enables to encapsulate single cells in microgels that are easily tunable (e.g. stiffness and bioactivity) due to the modularity of the UPy-building blocks and thereby creates a platform to investigate individual cells in a physiological relevant 3D synthetic microenvironment. For future experiments, UPy-microgels could be enriched with biological relevant UPy-additives.

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keywords: supramolecular, microgel, microfluidics, microenvironment, biomaterial

PS52
Perspectives For Future Innovation in Tendon repair (P4 FIT)

73296379029

A COMPUTATIONAL MODEL TO OPTIMIZE COMPONENTS AND OPERATIONAL PARAMETERS OF A NOVEL FLEXIBLE MECHANICAL STIMULATION BIOREACTOR

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An emerging feature of mechanical stimulation tissue engineering are flexible bioreactors that can move together with the actuation system. These have the potential of applying more physiologically relevant movement regimes than conventional systems. The development of such a bioreactor requires the optimal design and operational parameters. A mathematical model was developed to provide better insights into the system, by highlighting heterogeneous nutrient distribution due to the geometry, oxygen permeability, cell distribution, flow velocity or inlet location. Furthermore the aim was to find an optimum flow rate considering residence time, replenishment of nutrients and their effects on cell growth.
The chamber consists of 3D printed parts, a 3cm long filamentous scaffold and a lose membrane fit around it to make the system air-tight. There is one inlet and one outlet located on each opposite end of the chamber, through which media is continuously fed via a peristaltic pump.
We consider fluid flow and mass transport in the bioreactor chamber. The geometry consists of an outer cylinder, representing the scaffold, and a free flow area around it as the space between scaffold and membrane. We operate in a cylindrical coordinate system (r, theta, z) and assume axisymmetry, so that quantities do not vary with theta, and there is no flow in the azimuthal at z=0. The fluid enters the chamber from the inlet at a volumetric flow rate, carrying Glucose and Oxygen, and leaves at the outlet. We model the fluid as incompressible Newtonian of constant viscosity and density. Nutrients are fed via the fluid through the inlet and leave the system at the outlet. Nutrients diffuse through the scaffold and free flow area at different rates, given by individual diffusivity constants. Nutrient consumption and metabolite production is modelled by Michaelis Menten kinetics. Cells are located on the scaffold only, where they can consume nutrients, produce lactate multiply and spread. We assume cells are initially homogeneously distributed through the scaffold. Cell growth is modelled with a Monod-type kinetic equation. The availability of Oxygen and Glucose is needed for cell growth, whereas Lactate concentration slows down growth.
The multiphysics software COMSOLwas used to solve this model by coupling the porous and free flow module to the transport of diluted species in porous media modules.
Different flow rates, and initial cell numbers were modelled in a parametric sweep, to answer the question of what parameters will lead to sustained cell growth. To avoid depriving cells of oxygen too quickly, the introduction of a membrane permeable to gasses is tested.
The resulting simulations showed oxygen depletion and growth inhibiting lactate concentrations at low flow rates, and an optimum cell growth curve at intermediate flow rates. Oxygen delivery was greatly improved upon the introduction of the permeable membrane. There is ongoing experimental work to validate the results of this model.

keywords: Computational Model, Bioreactor,

52354532557

CONNECTING SCIENCE AND SOCIETY THROUGH EDUCATION AND PUBLIC ENGAGEMENT – A CASE STUDY FROM AN IRISH MEDICAL DEVICE RESEARCH CENTRE

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Introduction
The medical device sector relies on the detail-oriented and often cutting-edge research of fundamental scientists. This type of research requires public funding to survive. As such, engaging effectively and meaningfully with the public in the research undertaken, for people to understand and appreciate the importance of funding lab-based, discovery research is vital for continued public support and investment. However, scientists face considerable challenges in effectively and efficiently communicating the content and potential societal value of their endeavours.1 Research centres can support scientists to communicate the societal impact potential of their research activities and engage with public groups.2

This paper presents a case study of the education and public engagement (EPE) initiative at CÚRAM SFI (Science Foundation Ireland) Research Centre for Medical Devices. The centre is focused on the development of biomedical implants, therapeutic and diagnostic devices that address the needs of patients living with chronic illness. CÚRAM established an EPE initiative to support their researchers in science communication and public engagement. The programme is centred around three core pillars, connecting medical device science with the public through collaborations between scientists and filmmakers, educators and artists.

Methodology
Using a case study research methodology, due to its value in investigating contemporary context specific phenomena3, a retrospective exploration of the first five years (2015-2019) of the EPE initiative at CÚRAM was carried out. A combination of primary and secondary data sources were incorporated into the study, including interviews with participants, various evaluation efforts of the EPE team, annual reports, and supplementary materials. A logic model analysis approach was chosen to capture the breadth and depth of the programme's societal impact potential.4

Results
A number of novel strengths and challenges were identified across the three EPE pillars for communicating and connecting fundamental bench research to the public that funds it. The wide variety and novelty of engagement and collaboration strategies, with a focus on facilitating participants to cocreate the outputs of each project, was identified as key to the long term impact potential of the programme. These different pillars, in combination, offered a suite of approaches and strategies to connect sustainably with a widely diverse set of public audiences. Other strengths of the programme included high levels of scientist engagement, clear benefits of EPE for scientists, a growing network of collaborators and funders, the variety in public education and engagement strategies for different audiences, and the overall novelty of the programme. A number of challenges for the different stakeholder groups was also identified.

Conclusions
CÚRAM's EPE initiative provides a practical example of how life science-oriented research centres can support their research communities to engage meaningfully and sustainably with public groups, communicating the impact potential of medical device research to the public that ultimately funds it.

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keywords: Education, public engagement, science communication, medical device research

73296308044

FABRICATION OF ELECTROSPUN POLY(GLYCEROL SEBACATE) AND POLY(E-CAPROLACTONE) ALIGNED SCAFFOLDS: A COMPARISON BETWEEN THEIR MECHANICAL PERFORMANCE FOR TENDON TISSUE ENGINEERING APPLICATIONS

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Introduction. In the context of tissue engineering for regenerative medicine, the electrospinning technique consolidated itself as a valuable approach to fabricate tailored scaffolds. While its versatility lies in the possibility of controlling the process parameters, ambient parameters, and the properties related to the solvent system, the choice of the polymeric component itself results fundamental when designing a scaffold for a particular application. For tendon regeneration specifically, it is known the mechanical properties and alignment of the fibers play a major role in mimicking the features of native tendon, made of highly aligned and robust bundles of collagen fibrils. Poly(glycerol sebacate) (PGS) is a tough biodegradable polyester with elastomeric properties, easily synthesized through polycondensation of glycerol and sebacic acid; poly(ε-caprolactone) (PCL) is a semi-crystalline polyester widely employed in tissue engineering, with high miscibility potential with other polymers. Both polymers are biocompatible and bioresorbable, and their blend electrospinning can produce fibrous mats with promising mechanical properties. However, the optimization of the electrospinning process of this blend, especially in a benign solvent system1, and its application for tendon tissue engineering are mostly unexplored to this day2.

Methodology. PGS pre-polymer was synthesized and mildly crosslinked as reported elsewhere3. Mildly crosslinked PGS, PCL, and different ratios of PGS/PCL randomly oriented and aligned fibers were produced with a commercially available electrospinning setup in glacial acetic acid. The electrospinning parameters were an applied voltage of 15 kV, a tip-to-collector distance of 11 cm, and a flow rate ranging from 0.4 to 1.1 mL/h1. Randomly oriented and aligned fibers were collected on a drum rotating, respectively, at 500 and 1800 rpm. Morphological and chemical characterizations were carried out through Scanning Electron Microscopy and Fourier-Transformed Infrared Spectroscopy, degradation studies were conducted up to 14 days, and the mechanical properties were evaluated by standard uniaxial tensile testing.

Results. Homogeneous bead-free fibers were obtained. Characteristics absorption bands of PGS, PCL, and PGS/PCL fibers fabricated in presence of acetic acid were comparable to the ones found in literature. Pristine and blend fibers showed distinguishable degradation kinetics, attributable to the different degradation rates of PGS and PCL. Aligned fibers performed mechanically better than randomly oriented fibers, with PCL fibers having the highest ultimate tensile strength and strain at failure.

Conclusions. Aligned fibrous scaffolds from PGS, PCL and PGS/PCL were successfully produced

through green electrospinning; the fibers were homogeneous and free of defects. Their mechanical properties met the requirements for tendon tissue engineering, with the ultimate tensile strength and Young's modulus being comparable to the desirable values for the least demanding tendons, and the strain at failure being way higher than any native tendons, highlighting their potential use for tendon regeneration.

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keywords: scaffolds, tissue engineering, electrospinning, tendon regeneration, synthetic polymers

73296329057

IDENTIFICATION AND CHARACTERIZATION OF PORCINE-TENDON DERIVED STEM CELLS (TDSCS)

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Introduction
Restoring the normal structure and function of injured tendons is a major challenge in orthopaedics. The discovery of tendon-derived stem cells (TDSCs) provides a novel perspective to treat tendon injuries, which is expected to be an efficient approach to promote tendon repair and regeneration (Li et al., 2021).

Methodology
Isolation of TDSCs
The isolation of tendon derived stem cells (TDSC), both from the sheath and from fibres, was performed following a published protocol (Yang et al., 2018). In brief, tendon tissues were dissected from the Achilles tendon of a pig and washed with antibiotic. After washing, the tissue was cut into small pieces and digested with type II collagenase overnight at 37˚C. The enzymatic activity was neutralized with fetal bovine serum and tissue pieces passed through a 70 µm cell strainer to yield single-cell suspension and then centrifuged. The released cells were resuspended in L-DMEM; Lonza, 15% FBS, 1% Penicillin-Streptomycin-Amphotericin B solution, 1% L-glutamine, 0.4 ng/ml epidermal growth factor, 2.5 ng/ml basic fibroblast growth factor and 2.5 ng/ml stem cell factor. Cells were seeded at 50,000 cells/well seeding density and cultured at 37˚C in two different oxygen conditions (2% and 21%). After 48 h of initial plating, the cells were washed twice to remove remaining non-adherent bodies. After one week, when cells reached confluence, they were split at 5000 cells/cm2. Starting from passage P1, conditioned media (serum-free) was collected in order to isolate and characterize Extracellular Vesicles (EVs).
Isolation of Tenocytes
The isolation of tenocytes was performed using the same pig used for TDSC isolation, only by plating directly into 6-well plates small pieces of pig tendon fibres covered with DMEM, 10% FBS, 1% PSA, 1% L-glutamine and 1% Nonessential amino acids.

Results
TDSCs from tendon fibres displayed significantly enhanced rates of proliferation when compared to both TDSC from the tendon sheath and tenocytes. This was further influenced by culture in a physiological 2% O2 condition where all cells proliferated more rapidly than their air oxygen cultured counterparts.
Flow cytometry analysis was performed to confirm the expression levels of surface markers: CD14, CD19, CD34, CD44, CD45, HLA-DR, CD73, CD90, CD105. Subsequent molecular analysis confirmed the expression of tenocyte-specific markers: Scleraxis, Tenomodulin, and Thrombospondin 4, and of stem cells markers: Nanog, Oct-4 and Nestin. The multi-lineage differentiation potential of porcine TDSCs was then performed for osteogenic, chondrogenic and adipogenic induction media to confirm stem cell properties. The extraction and characterization of EVs from TDSCs and tenocytes was performed to confirm their cell-instructive-effect with protein quantification by Bradford assay, size distribution determination via Zetasizer, and a THP-1 based immunological model.

Conclusion

TDSCs from tendon fibres display an enhanced proliferative potential than those from sheath or tenocytes. Immunophenotype, molecular analysis, and differentiation potential are consistent across all cell types while EV functionality differences remain to be determined.

keywords: porcine, TDSCs, tenocytes, EVs

62825419005

LPS ENHANCES THE IMMUNOMODULATORY PROPERTIES OF AMNIOTIC EPITHELIAL STEM CELLS CONDITIONED MEDIA

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Introduction:
Tissue engineering has the purpose to find a resolutive treatment for unsolved pathologies, as tendon disorders, taking advantage of cell-free approaches [1]. Since tendinopathies have been related to persisting inflammation state, the application of immune regenerative strategies is getting attention by exploiting the immunomodulatory potential of stem cells and their derivates [2,3]. Amniotic Epithelial Stem Cells (AECs) represent a potential stem cell source due to their teno-differentiate [4] and immune modulating abilities both in vitro and in vivo [5]. In this context, this research aimed to obtain a conditioned media enriched with immunomodulatory factors by stimulating AECs with LPS [6] to be employed in the treatment of tendinopathies.

Methodology:
AECs isolated from the amniotic membrane obtained from ovine fetuses at mid gestation, were seeded under standard cultured condition [4]. At 70% of confluence, AECs were stimulated 1h with 1 μg/ml LPS (AEC+LPS) [6] and then serum free media was added for 24h before the collection of the samples. Unstimulated AECs were used as control (AEC). Real time-PCR (RT-PCR) was used to analyse anti-inflammatory IL10 and pro-inflammatory IL12 mRNA expression levels. The conditioned media (CMs) were collected to assess the profile of 40 immunomodulatory cytokines using Inflammation Antibody Array Membrane.

Results:
Preliminary data show that IL10 and IL12 mRNA were upregulated in AEC+LPS compared to AEC ($p<0.05$). Moreover, in AEC+LPS CMs it was registered an increased production of anti-inflammatory IL10, IL11 and IL12-p40, despite no changes of pro-inflammatory IL12 p70 (INF-γ stimulator) were detected. Also, a major production of chemokines associated with the recruitment of leukocytes (ICAM-1 and CXCL9), neutrophils (CXCL8) and eosinophils (EOXANTIN-2) and to the activation of immune cells (IFN-γ, IP-10/CXCL10) was observed. In contrast, stimulated AEC's CMs were characterized by a slight downregulation of some pro-inflammatory molecules such as IL-1α, TNF-α and IL17.

Conclusions:
These preliminary results demonstrate that AEC conditioned media enriched with immunomodulatory factors can be potentiated with LPS stimulation, suggesting its potential employment as immune inductive cell-free approach to be applied for tendon regeneration. Future biological tests with immune cells will be performed to confirm the immunomodulatory potential of the produced activated AEC conditioned media.

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keywords: Immunomodulation, AECs, secretome, LPS

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MICROFLUIDICS DEVELOPMENT OF POLYMERIC HYDROGEL MICROSPHERES FOR DRUG DELIVERY APPLICATIONS.

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Introduction: Musculoskeletal diseases include more than 150 pathologies and have been reported to affect 1.71 billion people worldwide (1). Among the different conditions described, the number of cases of chronic tendinopathy and tendon/ligament injuries has reportedly increased, mainly due to the rise of life expectancy in many countries (2). The currently used therapeutic tools mainly focus on the treatment of the associated symptomatology and, in the case of major injuries, surgery remains a common alternative, even though the complete recovery of the patient is highly difficult due to the risk of scar development, tendon adhesion, reduced joint movement, limited mechanical properties (1). Regarding chronic tendinopathy, tendon regeneration can be enhanced thanks to the administration of specific drugs such as different growth factors (3). Bearing this in mind, new therapeutical alternatives for the treatment of tendon injuries should be studied. This project focuses on studying polymeric hydrogel microspheres for the delivery of biological agents with tendon regenerative properties.

Methodology: Polymeric hydrogel microspheres are prepared using glass microfluidic technology (4). The formulation is prepared using two different precursor polymers: Norbornene functionalized 8-arm Polyethylene glycol (PEG) and Tetrazine functionalized 4-arm PEG. The obtained polymeric hydrogel microspheres are studied in terms of their size and variation coefficient. Subsequently, the formulation is morphologically studied using scanning electron microscope (SEM) and the rheological properties are studied. Moreover, the degradation behavior of the formulation is also assessed. The formulation is loaded with a model protein, such as bovine serum albumin (BSA) to study the entrapment efficiency and the release rate. Afterwards, the microspheres are loaded with different growth factors and, subsequently, in vitro cell viability and efficacy studies is performed in tenocytes.

Results: Monodisperse polymeric hydrogel microspheres with a lower size than 50 μm and a low variation coefficient are obtained. The microspheres have a high entrapment efficiency of biological compounds and a slow-release rate. The selected precursor polymers do not show any toxic effects.

Conclusion: In this study, we have developed a co-flow focusing microfluidic technique for the preparation of polymeric hydrogel microspheres via an emulsion method in mineral oil. The microspheres were used to encapsulate different drugs and release them following a slow process. This system is expected to release bioactive molecules and be loaded in an external hydrogel with other bioactive molecules and even stem cells in the future.

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keywords: Tendinopathy, Hydrogel, Microsphere, Microfluidics

31412717829

NATURAL-ORIGIN POLYMERIC SCAFFOLDS FOR TENDON ENGINEERING PRODUCED VIA DIFFERENT CROSSLINKING METHODS: MECHANICAL PERFORMANCE AND CHARACTERIZATION

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Introduction:
Gelatin is a biocompatible polymer derived from natural sources. Its low cost and non toxicity make it an attractive alternative in tissue engineered scaffolds. Chitosan, a biodegradable polymer obtained from chitin, is widely used due to its antimicrobial properties and chemical similarity to glycosaminoglycans (GAGs) found in native tissue. Cellulose nanocrystals (CNCs) are 10 nm wide, 150 - 300 nm long crystals derived from cellulose, and can function as a reinforcement to improve the mechanical properties of scaffolds. As well as being naturally derived, they exhibit no cytotoxicity.
When combined, gelatin and chitosan can produce biocompatible and biodegradable scaffolds, with applications in various tissues, however, crosslinking is a vital step in the production of these scaffolds. Not only is it a way of improving their biodegradation behavior, it also allows them to reach the appropriate values of strength and stiffness for tendon tissue applications.
The aim of this work is to analyze and compare the performance of a novel, all-natural polymer scaffold crosslinked using three different methods. Gelatin-chitosan scaffolds reinforced with cellulose nanocrystals (CNCs) are made via freeze drying and treated with the one of the following crosslinking agents: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), genipin and microbial transglutaminase (mTG).

Methodology:
10% gelatin solution and 3% chitosan solution are mixed in a 1:1 ratio, after which CNCs are added. The solution is cast into molds, cooled slowly to allow for gel formation and subsequently freeze dried. Crosslinking with genipin and EDC is carried out by soaking the scaffolds in a crosslinking solution for 24 hours, washing and freeze drying; crosslinking with mTG is carried out by adding the crosslinking agent to the polymeric solution[1].
Scaffolds are characterized via Scanning Electron Microscopy for pore size and contact angle for wettability. Tensile and compressive strength of the scaffolds are measured with an Instron Universal Testing system, after soaking the samples for 5 minutes in phosphate buffered solution. Porosity and water uptake measurements methodologies are described elsewhere[2].

Results:
Results show scaffolds with very high porosity (>90%) when non crosslinked, and slightly lower when crosslinked (>70% for all crosslinkers). Average pore sizes are found to be in the 150 - 300 µm range. Scaffolds exhibit hydrophilicity with contact angles lower than 80°; tensile strength is highest for scaffolds crosslinked with genipin, followed by EDC.

Conclusions:
All-natural polymeric scaffolds were successfully produced and crosslinked with 3 different agents. Chemical crosslinking was achieved, as demonstrated by an increase in the material's mechanical properties. Scaffolds crosslinked with genipin and EDC showed encouraging values of tensile strength. Further analysis is required to determine the feasibility of these crosslinked scaffolds for tendon engineering.

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keywords: scaffolds, natural polymers, tendon tissue engineering

62825423466

OVINE ADIPOSE DERIVED STEM CELLS AS POTENTIAL STEM CELL SOURCE FOR TENDON REPAIR

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Introduction:
Stem cells-based therapy has emerged as a new strategy within the field of regenerative medicine, proving its effectiveness in tendon regeneration [1]. Amongst the stem cell sources, Adipose-Derived Stem Cells (ADSCs) can differentiate towards the tenogenic lineage and modulate the inflammatory environment [2]. However, the mechanisms through which ADSCs promote tendon healing in vivo are barely understood. This research aims to study the ovine ADSCs properties to be used as stem cells-based therapy for tendon regeneration.

Methodology:
Sheep ADSCs from tail region were isolated according to the protocol developed within FAT STEM laboratories [3]. Isolated ADSCs were then expanded until passage 6 (P6) in growth medium (GM) consisting of a-MEM supplemented with 20% FBS [1]. Cells proliferation capacity was assessed employing an MTT assay by culturing ADSCs, from P1 and P5, at 5x103 and 10x103 cell/ml while cell morphology was evaluated under an inverted microscope after 24h, 48h, and 72h culture.

Results:
The obtained preliminary results show that ADSCs changed morphology and proliferation capacity in culture. Cells at P1 displayed a more elongated, fusiform shape with bigger nuclei, while cells at P5 presented a more irregular shape with smaller nuclei. MTT assay results demonstrated differences in ADSCs proliferation used from P1 and P5. ADSCs from P1 exhibited a similar proliferation rate after 24h and 48h, 0.102 ± 0.001 and 0.105 ± 0.027, respectively. Instead, at 72h, ADSCs proliferated exponentially with significant values compared to 48h, with a faster proliferation rate with 10x103 cell/ml (0.381 ± 0.017 vs. 0.224 ± 0.008, $p<0.05$). In ADSCs at P5, the proliferation was concentration-dependent since cells seeded at high concentration of 10x103 cell/ml showed a significant proliferation rate ($p<0.05$). compared to low concentration. ADSCs from P1 proliferated faster between 48h and 72h while those of P5 showed a higher proliferation rate between 24h and 48h. These results were confirmed by analyzing doubling times that were about 17h (P1) and 13h (P5) for 24h to 48h culture, and about 13h (P1) and 22h

(P5) for 48h to 72h culture independently to cells' concentrations.

Conclusion:
Preliminary data showed that ADSCs exhibited differences in morphology and proliferation rate dependent on their passages. Different markers including CD44, CD34, CD31, and CD45 will be analyzed through flow cytometry for better characterization of ADSCs phenotype. The possible changes in ADSC's metabolic activity will be also assessed through beta-galactosidase assay to test the senescence hypothesis. In addition, the teno-inductive potential of ADSCs will be tested in vitro by co-culturing cells with fetal tendon explants.

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keywords: Regenerative medicine, ADSCs, tendon healing

31412712306

POLYMERIC-BASED NANOPARTICLES FOR TENDON INFLAMMATION TREATMENT

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INTRODUCTION
Tendinopathy (also called tendinitis or tendinosis) is a tendon disorder resulting from repetitive motion in the affected area or during athletic activity.It is related to pain and swelling in the affected area, with decreasing mechanical strength and movement. [1] With the rapid growth of an aging population, there is an increasing incidence of chronic tendinopathy.Current therapeutic options are limited and usually used only for symptomatic relief; although surgical treatment can alleviate some symptoms, [2] the long-term effect (e.g., tendon adhesion, decrease of mechanical strength and risk of repeated fractures) are unsatisfactory.In these last years, Nanotechnology earned attention for the development of diagnostic and target drug delivery based on a specific design. [3] Nanoparticles (NPs), which have small size, can improve bioavailability, timed/controlled release of drugs and have more precise targeting to the intracellular delivery. [3]
In this work, we have developed an innovative approach aiming at treating tendinopathy, and solving the problem of the inflammation in tenosynovitis and tendonitis, while avoiding the fibrosis during the healing process of the tendon.

METHODOLOGY
Acetalated dextran-based NPs are loaded with curcumin (a drug with anti-inflammatory and anti-infective action) and coated with Tannic Acid-Iron (III), two compounds which have shown to have anti-fibrotic effects when combined together. [4]Microfluidics was used for improving the uniformity of the particles size. [5]
The NPs were produced by nanoprecipitation in a glass-capillary microchip.The size and zeta (ζ)-potential were measured by dynamic light scattering, and the loading degree (LD) and efficiency of encapsulation (EE) determination were performed by high pressure liquid chromatography. The stability was also investigated in aqueous solution,+4 °C and room temperature. Furthermore, the morphology of the nanosystem was studied by acquiring transmission electron microscopy (TEM) images and the biocompatibility was screened in tenocytes.

RESULT AND DISCUSSION
The prepared NPs showed a size below 300 nm and ζ-potential of -40 mV, which were characterized by a specific LD and EE of curcumin, respectively around 5% and 50%.The storability has shown good stability over the time at +4 °C compared to the samples kept at room temperature.
Moreover,we investigated also the morphology of these particles by acquiring TEM images,and these results showed uniformity in size regarding the loaded NPs, and the presence of an irregular coating on the surface for the coated ones.The cell viability results proved a good compatibility of this system in tenocytes.

CONCLUSION
Here,we developed an innovative and easily scalable drug-loaded nanosystem,which has

shown a good stability and biocompatibility in vitro.The nanosystem will be next tested for the treatment of tendon inflammation.

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keywords: tendinopathy, nanoparticles, microfluidics, regeneration

73296315524

STRUCTURAL AND IMMUNOLOGICAL CHANGES DURING SPONTANEOUS HEALING IN ACHILLES TENDONS MICE MODELS.

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Introduction:
Recovery after tendon injuries remains a challenge for the orthopaedics to be solved due to the frequently poor clinical outcomes (1,2). To date, the cellular implications as well as the structural changes in tendinopathy are not fully known, especially concerning the link between inflammation and the early stages of tendinopathy (3). The aim of this research was to assess the structural changes and immune response that occur during spontaneous tendon healing in mice models.

Methodology:
Animal experimentation was authorised by the Italian Ministry of Health (183/2021). Under general anaesthesia, a bilateral incision of approximately 0.5 mm was induced on mice Achilles tendons. Healthy mice tendons were used as control. After 28 days postoperative, samples were used to perform Hematoxylin-Eosin, Alizarine Red staining, and immunohistochemistry (IHC) assays to assess the expression of collagen type 1 (COL1), collagen type 3 (COL3), von Willebrand Factor (FvW, an endothelial cell marker) and immune markers such as CD68 (pan-macrophage marker), CD86 (M1 macrophage), CD206 (M2 macrophage) markers. The samples were examined under light and fluorescence microscopes.

Results:
To understand the structural changes that occur during spontaneous tendon healing, the diseased mice tendon was compared to the healthy tendon tissue structure. Healthy tendons are characterized by hypocellularity, low vascularity and COL1 fibres parallelly organized along the longitudinal axis of the tissue. Furthermore, IHC demonstrated the absence of

the immune cells markers within the healthy tendon. However, during spontaneous tendon healing, a disorganized hypercellularity was noted within the lesioned tendons accompanied by the presence of chondrocyte-like cells surrounded by a basophilic structure. The presence of chondrocytes was then confirmed by performing Alizarine Red staining from which chondrocyte-specific structures were identified, demonstrating the presence of calcium deposits around the chondrocytes and throughout the lesioned areas. Additionally, there was a neo-formation of irregular collagen fibres, characterized by a predominant COL3 expression compared to COL1 during the early stage of spontaneous tendon healing, demonstrating an incomplete substitution of COL3 by COL1.Moreover, blood vessels within the healing tendon were assessed by analysing FvW. Compared to the healthy tendon, an increased number of blood vessels irregularly distributed was observed, especially within calcification areas. Finally, the analysis of immune markers, CD68, CD86, and CD206, revealed the presence macrophages within the lesioned tendon tissues.

Conclusions:
The preliminary results demonstrated structural differences between healthy and spontaneous tendon healing tissues. The majority of the spontaneous healing tendon samples presented chondrocyte-like cells and different stages of calcification processes, as well as major structural alteration, which might confirm that spontaneous tendon healing does not offer a full recovery and regeneration of tendon tissue. These results will be confirmed by analyzing the gene expression profiles of tendon and immunomodulatory genes and will be further compared with tendon healing after stem cell transplantation.

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keywords: Tendinopathy, immunomodulation, mice Achilles tendon, ECM remodelling

83767224966

TENOGENIC DIFFERENTIATION INDUCED BY COOPERATIVE GROWTH FACTORS

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In tendon biology, considerable progress has been made in identifying tendon-specific genes. However, despite the comprehensive knowledge of the tendon mechanical function, neither the ontogeny of the tenogenic lineage nor signalling cascades are fully understood (Citeroni et al., 2020). For this reason, it is important to define the main actors in the developmental pathway that induces the sequential differentiation of MSCs into Tendon Progenitor Stem Cells and then Tenocytes. The main objective of this study was to define the best and-logic system in which the biological signal power and its presentation cooperate to drive specific differentiation paths.

TSPCs and tenocytes were isolated from pig-derived tendon tissue (Yang et al., 2018). MSCs were obtained from human bone marrow (Kay et al., 2015). We showed that TPSCs, similarly to MSCs and differently than tenocytes, have stem cell characteristics such as clonogenicity, multipotency and self-renewal capacity. Stem cells and tendon-specific markers, Scleraxis, Oct-4, Nanog, Nestin, Tenomodulin and Thrombospondin-4 were investigated in parallel in all three cell lines to establish cell-specific peculiarities in their genetic profiles. Cell characterization and functionality were evaluated through surface marker expression, analysed via flow cytometry. BMP12/BMP13/BMP14 were used as media supplementation, individually and in combination, to induce hBMSCs and TPSCs differentiation, and the expression of tenolineage transcription factors and matrix proteins was compared.

hBMSCs expressed surface markers CD73, CD90 and CD105 and stained negative for CD14, CD19, CD34, CD45 and HLA-DR. Multi-lineage differentiation potential of hBMSCs was demonstrated for osteogenic, chondrogenic and adipogenic induction media. Conversely, when extracted from the tendon, TPSCs rapidly lost their differentiation potential starting to express more tendon-related markers. The marker CD44 has been proved to be a discriminative element between TPSCs and tenocytes. The independent exposure of three different growth factors outlined the time-dependent role of any growth factor in any specific phase of the differentiation, allowing us to optimize their synergic effect.

MSCs can improve tissue regeneration and healing by three main mechanisms: differentiation, promotion of angiogenesis, and inflammatory response control. All these mechanisms can be induced by specific growth factors. An in vitro evaluation of tenogenesis in two-dimensional culture is always based only on relative changes in the set of molecules expression, for this reason, there is no unequivocal method to recognize completed tenogenic differentiation.
We selected a specific subset of BMPs growth factors that are specifically recognized for their ability to induce tenogenic differentiation. These growth factors have been selected because they represent different areas of growth factor-mediated effect and so they allow us to retrace the developmental tenogenic pathway. The use of bioactive molecules in tenogenesis may have critical implications for the early phase of tendon tissue engineering. Indeed, a better understanding of the effect of bioactive molecules in tenogenesis will lead to more complex systems in which the transcriptional differentiation could be supported by composite living

fibre able to improve the synaptic effect related to cells capability to sense each other. Future challenges will involve the design of three-dimensional systems capable to reproduce the native environment of tendon tissue.

keywords: tendon progenitor stem cells, human mesenchymal stem cells, growth factor supplementation, tenogenesis, GDF5/GDF6/GDF7 controlled delivery, tissue engineering.

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THE EFFECT OF BLENDING NATURAL POLYMERS WITH POLYCAPROLACTONE NANOFIBROUS SCAFFOLDS AND EVALUATION OF THEIR POTENTIAL FOR TENDON REGENERATION

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Introduction: As a tissue engineering approach, synthetic and natural polymer composites are explored to create tissue scaffolds that provide requirements such as biocompatibility, biomimetic interface, mechanical properties close to native tissue, and promotion of cell attachment and differentiation. Blends of natural and synthetic electrospun fibers can provide excellent combinations to obtain both mechanical and bioactive properties simultaneously.

Methodology: In this study, we synthesized nanofibrous tissue scaffolds made up of polycaprolactone (PCL), and blends with either chitosan (PCL/C) or gelatin (PCL/G) in benign solvent systems for evaluation of their potential as a scaffold for tendon tissue regeneration. The electrospinning process parameters were tailored towards fiber diameter and morphology which should be optimized for cell attachment, differentiation, and proliferation. The nanofibrous scaffolds were characterized in terms of fiber morphology and size, chemical composition, biodegradability, hydrophilicity, and biomechanical properties by tensile test measurement.

Results: We demonstrated that the addition of natural polymers yields fibers in nanosize with significantly lowered fiber diameters, and mechanical properties consistent with those displayed by native tendon tissue. The combination of natural and synthetic polymers has also reduced the degradation time together with the water contact angle of the nanofibrous membrane surfaces.

Conclusion: The combination of natural and synthetic polymers in the production of electrospun nanofibrous scaffolds has the potential to promote tendon tissue regeneration. The present results must be however supported by further in vitro and vivo studies and testing.

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keywords: electrospinning, tendon regeneration, polycaprolactone, polymer blends

PS53
Prospects and Challenges in Biological Therapies for Tendon Regeneration

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HYPOXIC AND INFLAMMATORY TRIGGERS IN THE DEVELOPMENT OF TENDON PATHOLOGIES: INSIGHTS ON TENOCYTE BEHAVIOR USING A MAGNETIC CELL SHEET MODEL

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Introduction: Tendon injuries represent a significant personal and socioeconomical burden whose medical treatments remain insufficient to restore normal functionality. Tendon healing is often impaired, contributing for the lesion severity, risk of re-ruptures and ultimately tendon dysfunction[1]. Persistent inflammatory cues have been associated to the development of tendon pathologies, and to changes in tissue oxygen levels contributing to tissue hypoxia. In turn, hypoxia regulates inflammatory and fibrotic pathways through oxygen-sensitive pathways with impact in the quality of the tissue[2].
Thus, the interdependent relationship between hypoxia and inflammation may be clinically relevant for the pathogenesis of inflamed tendon injuries. Pulsed electromagnetic field (PEMF) has been investigated to modulate inflammatory cues expressed by human tendon cells (hTDCs) [3], and to reduce hypoxia effects decreasing pro-inflammatory cytokines (TNFalpha, IL-6, IL-8) in neuron-like and microglial cells[4].

Methodology: In this work we propose to explore the role of hypoxia in IL-1B-primed-hTDCs, and the influence of PEMF over hTDCs exposed to hypoxia. We use a magnetic cell sheet (magCSs) model made of hTDCs and magnetic nanoparticles, previously established[5], enabling the contribution of hTDCs and hTDCs-matrix interactions for cell behavior. Specifically, this study aims to assess i) the response of magCSs, under a permanent well-array magnet, to an oxygen tension (OT) of 1% and 2% after 1h, 4h, and 6h of exposure,in a hypoxic chamber; ii) the hypoxia effect in IL-1B-treated-magCSs (1ng/mL as established in[3]), and iii) the influence of an external PEMF (5Hz, 4mT, 50% duty cycle) on IL-1B-treated-magCSs in hypoxic environments.

Results: Our results show that HIF-1alpha and HIF-2alpha increase with time of exposure to OT in IL-1B-treated-magCSs, however in Control-group (unstimulated magCSs) this trend is not observed. Regarding the OT %, it was demonstrated a significant increase after exposure to 2% OT for 6h in IL-1B-treated-magCSs compared to 1% OT and Control-group.
The expression of TNFalpha, IL-6 and IL-8 was enhanced in hypoxic environments, independently of the time or OT. When IL-1B-treated-magCSs exposed to hypoxia are PEMF-stimulated, there is a decrease in the expression of these genes, and an increase in anti-inflammatory IL-4 and IL-10 and in HIF-1alpha and HIF-2alpha compared to Control-group.
The cell nuclei aspect ratio indicative of cell alignment was significantly lower in IL-1B-treated-magCSs exposed to 1% OT in comparison to 2% OT, independently of the hypoxia exposure time. Moreover, IL-1B-treated-magCSs exposed to hypoxia showed the highest values under PEMF-stimulation (>1), suggesting that PEMF favors cell alignment in magCSs-constructions.

Conclusions: Overall, low oxygen tension enhances the inflammatory profile and hypoxia-associated genes in IL-1B-treated-magCSs. PEMF modulates the response of magCSs exposed to hypoxia favoring the expression of anti-inflammatory genes even after exposure to adverse environmental conditions provided by low oxygen tensions and inflammatory triggers.

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keywords: Hypoxia, Magnetic actuation, Inflammation, Magnetic cell sheets, Tendon cells

52354549705

INTERPLAY OF TGFB3- AND RHO/ROCK SIGNALING IN TENOGENIC DIFFERENTIATION

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Multipotent mesenchymal stromal cells (MSC) represent a promising therapeutic tool for tendon regeneration. They are capable of tenogenic differentiation, which is one of the mechanisms contributing to the regenerative effect of MSC after transplantation into tendon lesions and is furthermore required for tissue engineering approaches. Tenogenic differentiation is most typically induced by growth factors such as transforming growth factor (TGF)-β3, which involves intracellular activation of smad signalling [1]. On the other hand, tenogenic differentiation was shown to be induced by mechanical stimuli and extracellular matrix (ECM) components, for which rho/ROCK signalling was demonstrated as essential [2]. In addition, previous studies demonstrated an alteration of TGF-β3-induced tenogenic differentiation during culture on tendon ECM [3]. This suggests that there is an interaction between the different tenogenic induction mechanisms and their signalling pathways. Phosphorylation of the smad2/3 molecule at different phosphorylation sites represents a possible interface. While TGF-β3 is known to phosphorylate the carboxy-terminal region of smad2/3 and thereby activate it, some studies described an inhibitory phosphorylation of the linker region of smad2/3 by the rho/ROCK pathway. Here, the interplay of rho/ROCK and TGF-β3/smad signalling in tenogenic differentiation were investigated, with the smad2/3 molecules as possible interface.
Primary human adipose-derived MSC were cultured as monolayers, on equine tendon-derived decellularized scaffolds or on 3D collagen I gels. ROCK was inhibited by adding Y-27632 (10 µM) to the culture medium 2 h before further stimulation, then tenogenic differentiation was induced by adding TGF-β3 (10 ng/ml). Control cells were cultured accordingly, without Y-27632 and/or without TGF-β3. MSC were analyzed at different time points by real-time RT-PCR, immunofluorescence and western blot analysis.
TGF-β3 stimulation caused an increase in gene expression of the tendon marker scleraxis under all culture conditions. The strongest upregulation occurred when TGF-β3 and ROCK inhibition were combined [4]. Translocation of smad2/3 and scleraxis to the nucleus were observed accordingly by immunofluorescence staining. Furthermore, preliminary western blot analyses indicated a reduced linker region smad (S245/250/255) phosphorylation upon ROCK inhibition. ROCK inhibition was confirmed by disruption of the actin cytoskeleton.

In conclusion, the results showed that ROCK inhibition promotes the TGF-β3/smad2/3 axis, which might due to reduced inhibitory phosphorylation of the smad molecule in the linker region.

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keywords: tenogenic differentiation, transforming growth factor β3

94238132884

REPARATIVE CAPACITY OF EX VIVO DEVELOPED SCAFFOLD-FREE 3D TISSUE EQUIVALENTS FOR TENDONS

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Introduction
Tendon injuries are difficult to heal because they do not regenerate by restitution but by the formation of a fibrotic scar. This scar tissue has poorer mechanical properties and often leads to long-term pain, discomfort and disability in movement. The particular physiology of tendons (hypovascular, hypocellular) and their structural, mechanical properties currently make it difficult to achieve complete and permanent repair of the damaged tissue using available treatments, especially when a complete tear is present. Despite multiple efforts, treatment options for tendon injuries are limited, which has led to the development of alternative therapies, such as the administration of growth factors, the use of stem cells, or tissue engineering of matrix-associated equivalents of tendons. To date, there are no techniques of restoring the original native structure of fully injured or ruptured tendons. One of the most promising methods being developed at the moment is the procedure of tissue engineering. It involves the production of tissue equivalents from the patient's own cells in the laboratory and their subsequent transplantation for safe and complete recovery of the damaged tissue.

Methodology/ Results
The challenge is to determine which cell type both differentiates into tendocyte-like cells in vitro and forms three-dimensional tendon equivalents ex vivo and how they mature. Since the main player of tendo tissue is fibrocytes, we focused on this cell type. Equine cells were targeted as the initial model for the project. Fibrocytes were isolated from equine lumbar region using skin punch (diameter 3mm) and propagated in monolayer cultures. Passage 3 cells were used for the preparation of tendon regenerates. Then, the monolayer cells were transferred to three-dimensional state. The plan was to roll several detached monolayers into each other to form the shape of a suture yarn. The implant created in this way is fixed to an elastic silicone surface by means of stainless steel pins. In this way, 3D constructs can be placed in a tense state in which the elastic subsurface is stretched. The continuously applied tension provides the newly synthesized ECM molecules of the tissue regenerate with a force vector along which they can align in the direction of the stretch, allowing the structure and morphology of tendogenic tissue to form. The fabricated tissue equivalents were studied biomechanically, histologically, and biochemically.

Conclusion
The initial results of the generated tendon regenerates show a promising perspective in further application as an in vitro model as well as in in vivo treatment.

keywords: Tissue engineering, tendon, scaffold-free, mechanical stimulation

83767281099

TISSUE ENGINEERED TENDON NANO-CONSTRUCTS FOR REPAIR OF CHRONIC ROTATOR CUFF TEARS IN LARGE-ANIMAL MODELS

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Chronic rotator cuff tears (RCTs) are one of the most common injuries of shoulder pain. Despite the recent advances in surgical techniques and improved clinical outcomes of arthroscopically repaired rotator cuffs (RCs), complete functional recovery—without retear—of the RC tendon through tendon-to-bone interface (TBI) regeneration remains a key clinical goal to be achieved. Inspired by the highly organized nanostructured extracellular matrix in RC tendon tissue, we propose herein a tissue engineered tendon nano-construct (TNC) for RC tendon regeneration. When compared with two currently used strategies (i.e., transosseous sutures and stem cell injections), our nano-construct facilitated more significant healing of all parts of the TBI (i.e., tendon, fibrocartilages, and bone) in both rabbit and pig RCT models owing to its enhancements in cell proliferation and differentiation, protein expression, and growth factor secretion. Overall, our findings demonstrate the high potential of this transplantable tendon nano-construct for clinical repair of chronic RCTs.

keywords: Chronic rotator cuff tears, large animal model, tendon nano-construct, tendon-to-bone interface, tendon regeneration

PS55
REMODELing the Future: next generation of organoid models for biomedicine

41883603786

GROWTH AND DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC)-DERIVED KIDNEY ORGANOIDS USING FULLY SYNTHETIC PEPTIDE HYDROGELS

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Human induced pluripotent stem cell (hiPSC)-derived kidney organoids have prospective applications ranging from basic disease modelling to personalised medicine. However, there remains a necessity to refine the biophysical and biochemical parameters that govern kidney organoid formation. Differentiation within fully controllable and physiologically relevant 3D growth environments will be critical to improving organoid reproducibility and maturation. Here, we matured hiPSC-derived kidney organoids within fully synthetic self-assembling peptide hydrogels (SAPHs) of variable stiffness (storage modulus, G′). The resulting organoids contained complex structures comparable to those differentiated within the animal-derived matrix, Matrigel. Single-cell RNA sequencing (scRNA-seq) was then used to compare organoids matured within SAPHs to those grown within Matrigel or at the air-liquid interface. A total of 13,179 cells were analysed, revealing 14 distinct clusters. Organoid compositional analysis revealed a larger proportion of nephron cell types within Transwell-derived organoids, while SAPH derived organoids were enriched for stromal-associated cell populations. Notably, differentiation within a higher G’ SAPH generated podocytes with more mature gene expression profiles. Additionally, maturation within a 3D microenvironment significantly reduced the derivation of off-target cell types, which are a known limitation of current kidney organoid protocols. This work demonstrates the utility of synthetic peptide-based hydrogels with a defined stiffness, as a minimally complex microenvironment for the selected differentiation of kidney organoids.

keywords: Human Kidney Organoids, Fully Synthetic Matrix, scRNA Sequencing

20941830609

UTILITY OF GELATIN METHACRYLOYL (GELMA) HYDROGELS AS TUNEABLE BIOPHYSICAL SCAFFOLDS FOR THE DERIVATION OF HIPSC-DERIVED KIDNEY ORGANOIDS

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The generation of human induced pluripotent stem cell (hiPSC)-derived kidney organoids have facilitated novel insights into renal developmental processes and have the potential to provide personalised treatment strategies for patients with end-stage renal disease. Traditional protocols for the directed differentiation of hiPSC-derived kidney organoids have predominantly focused on biochemical cues to specify hiPSCs towards a mesonephric lineage, but have not considered the influence of the surrounding biophysical properties of the extracellular environment on organoid formation. We hypothesised that Gelatin methacryloyl (GelMA), a derivative of collagen with mechanically amendable hydrogel stiffness profiles, could be used to investigate the influence of extracellular matrix stiffness on kidney organoid specification. hiPSC-derived kidney organoids were differentiated within photo-crosslinked GelMA hydrogels of defined mechanical strengths. Enrichment of renal cell types in response to the various mechanical microenvironments was subsequently investigated using immunocytochemistry, qRT-PCR, transmission electron microscopy (TEM) and histological staining methods. Rheological analysis of formulated hydrogels confirmed the generation of matrices with distinct stiffness (Young's Modulus, G') profiles. Hydrogels comparable to the stiffness of the gastrulation-stage embryo (G' = 400 Pa) and adult human kidney tissue (G' = 5-8 kPa) were generated. Importantly, the mechanical properties of the hydrogels showed remarkable stability even with prolonged time in culture. PCNA proliferation and cleaved caspase-3 apoptotic staining of organoids embedded within scaffolds demonstrated high cell proliferation and viability in the hydrogel conditions at day 24 of differentiation. The formation of glomerular, proximal tubular and distal tubular structures, supported by basement membrane and interstitial cells were confirmed in conditions using immunofluorescent imaging. Interestingly, qRT-PCR analysis revealed significant upregulation of nephron-associated genes (including PAX8, NPHS2, NPHS1, SLC3A1 and AQP1) in organoids differentiated within extracellular environments that approximated human kidney tissue, when compared to organoids differentiated within the much softer microenvironments. Our results illustrate the influence of the extracellular environment on appropriate cell fate determination and propose GelMA hydrogels as faithful extracellular supports for the specification of hiPSC-derived kidney organoids.

keywords: Kidney Organoids, Gelatin Methacrylol, Stiffness, Differentiation

PS56
Skin wound healing in 2022: where basic science meets clinical needs

62825456286

”ACTIVELAYERS” DEVELOPMENT OF MULTI-COMPONENT FILMS AS A PLATFORM TECHNOLOGY FOR APPLICATIONS IN WOUND HEALING AND SURGERY.

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“ActiveLayers” is a platform technology for building medical multilayer films based on layer-by-layer technology. It is characterized by its biocompatibility, environmental friendliness, modularity and multi-functionality where it can be optimized and adjusted to treat several medical problems such as; chronic wounds, non-healing bone fractures, periodontal regeneration and postoperative adhesions of organs. “ActiveLayers” acquires its uniqueness being the first-to-be product on the market based on this technology. Since the main challenge that prevents this technology from being on the market; is the transfer from research laboratories to industry due to the lack of effective upscaling options, we are working hard in this project to solve this problem. For the short term, we will be offering a new machine that enables high-throughput screening of LbL biomaterials, as well as, industry scale production of wound healing membranes and other customized medical membranes.

keywords: ActiveLayers, Medical membranes, Multi-functional, Biocomatiple, LbL based products

41883609306

ASSESSING THE IMPACT OF COMMON ANTISEPTICS FOR CLINICAL USE IN SKIN CELL LINES AND BIOENGINEERED AUTOLOGOUS SKIN SUBSTITUTES: AN IN VITRO STUDY

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Introduction
In recent years new therapies, such as skin cell lines injection and bioengineered autologous skin substitutes (BASS) technology, have emerged to promote re-epithelialization of damaged areas such as cutaneous wounds and ulcers or to treat patients with severe burns. Antiseptics are commonly used during wound healing to avoid serious infections, but some of them could have the opposite effect by delaying the healing process due to their apparent cytotoxicity against skin cells. Further studies on viability against standard procedures for wound healing and treatment protocol optimization are necessary for the improvement of BASS technology for clinical use. The aim of this study is to evaluate the effect of common antiseptics for clinical use in human fibroblasts and keratinocytes cell lines and in BASS, focusing on cell proliferation and viability, wound closure evaluation and inflammatory cytokine secretion as well as, epithelium and skin barrier integrity, in order to establish the least harmful treatment for wound care.

Methodology
The cytotoxicity of five antiseptics (ethanol, chlorhexidine digluconate, sodium hypochlorite, povidone iodine and polyhexanide) was evaluated in this in vitro study on human fibroblasts and keratinocytes and on BASS. Treatments were applied every 48 hours for 14 days to each cell type culture and for 16 days to BASS. To determine the cytotoxicity of the different antiseptics on skin cell lines, cell viability (Live/Dead®) and cell proliferation (AlamarBlue™) assays were performed on cell monolayers. The impact on cell migration capacity was also evaluated with a wound closure assay. For BASS, keratinocytes and dermal fibroblasts were isolated from skin samples and integrated into hyaluronic acid-based substitute. To determine the impact of treatments on BASS, cell viability (Live/Dead®), cytokine secretion (ELISA) and skin barrier integrity (Transepidermal Water Loss, TEWL) were evaluated.

Results
Chlorhexidine digluconate and ethanol significantly reduced the viability of keratinocytes and also inhibited cell migration compared to other treatments. For fibroblasts, povidone iodine followed by chlorhexidine digluconate significantly reduced cell viability. Wound closure assay showed that povidone iodine also inhibited cell migration. Likewise, sodium hypochlorite was the least detrimental to both cell types. Regarding BASS treatment, sodium hypochlorite was the only treatment that showed a high cell viability percentage throughout the evaluation time compared to other antiseptic treatments, as well as a similar cytokine secretion pattern as control BASS. No significant differences were found regarding epidermal barrier function.

Conclusions
This study revealed how antiseptics commonly used in clinical practice have high cytotoxicity on skin cell lines even at low concentrations, which can slow down the healing process by affecting cell viability and migration capacity. If the epithelial integrity is affected, the wound healing process can be altered, so the information gathered in this study may be useful in selecting the most suitable antiseptic after treatment with new emerging therapies to prevent infections. Interestingly, the findings of this research point towards sodium hypochlorite being the most suitable antiseptic treatment for BASS post-transplantation wound care.

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keywords: antiseptics, bioengineered artificial skin substitute, cytotoxicity, regenerative medicine, wound healing

20941858386

DERMAL RECONSTITUTION VIA POLYVINYL ALCOHOL/COLLAGEN FIBROUS MAT LOADED WITH EPIGALLOCATECHIN 3-GALLATE /CHITOSAN NANOPARTICLE

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Electrospun fiber comprising bioactive substrates has potential to implant into the wound site as a reliable therapeutic approach in tissue regeneration. Here, electrospun polyvinyl alcohol conjugated tyramine (PVA-Tyr) and collagen (Col) fibrous mat containing chitosan nanoparticle loaded with epigallocatechin 3-gallate (NCs-EGCG) developed and the composite was applied to evaluate in vivo wound healing ability of fabricated wound patch. The synthesized PVA-Tyr and Col were electrospun and crosslinked through peroxidase reaction in presence of vaporized H2O2 as an electron donor which covalently proceeded conjugation of phenolic groups and could develop hybrid fibrous mat in stable structure and uniform shapes. The EGCG as anti-oxidative/inflammatory substrate was encapsulated efficiently in NCs and released in a sustained manner. The hybrid fibers seeded with adipose-derived stem cells presented appropriate biocompatibility from biophysical and biochemical viewpoints and in following wound healing ability in a full-thickness excisional animal model. Fourier transform infrared spectroscopy (FTIR) confirmed all typical absorption characteristics of PVA-Tyr and Col as well as NCs and EGCG. The results showed the perfect hydrophilic/hydrophobic ratio and good mechanical and structural characteristics including shape uniformity and porosity. Interestingly, the cellular attachment and proliferation on the PVA-Tyr/Col fibers containing NCs-EGCG were higher than in control samples. The results showed that after 14 days, the wounds treated with the designed composite structure dressing achieved a significant closure of nearly 97% compared with the sterile gauze, as control, which showed nearly 74% of wound closure. The histological analysis of the hybrid fibrous patch could be suggested the applicability of this structure as suitable skin substitutes to repair injured skin.

keywords: Poly Vinyl Alcohol/ Gelatin conjugated phenolic moieties; fibrous mat; Sustained release of methylprednisolone; Spinal Cord Injury

52354538577

DESIGN OF A COMPOSITE WOUND DRESSING: COMBINING ELECTROSPUN GELATIN FLEECES AND FREE-STANDING LBL FILMS

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Introduction
Chronic skin wounds place a high burden on patients and health systems. In this study, we developed a composite of an electrospun fleece and a freestanding multilayer film, to combine their respective advantages.

Methodology
A gelatin solution was electrospun and the resulting fleece partially crosslinked in formaldehyde vapor. Spray coating was employed to build up a free-standing film directly on top of the crosslinked electrospun material. First a bonding layer-by-layer (LbL) system of oxidized hyaluronic acid (oxHA) and chitosan and afterwards a bulk LbL-system of alginate and chitosan as alternating polyelectrolytes were combined. The result is a composite with an electrospun fleece at the underside and a free-standing LbL system on top. Afterwards the composite was crosslinked with genipin. The uncrosslinked and genipin crosslinked composites were compared to the individual fleece and free-standing film. The structure, layer growth and swelling properties were characterized by confocal microscopy, profilometry and nano-tomography. Dynamic mechanical analysis was performed to evaluate the mechanical properties. Additionally, cell experiments with normal human dermal fibroblasts (NHDF) were performed to test biocompatibility, cell adhesion and proliferation. Proliferative assays were accompanied by immunohistochemical staining.

Results
The fleece and film were successfully combined with proficient bonding provided by the crosslinking of oxHA with gelatin. On the bottom side of the composite, the porosity provided by the fibers of the electrospun fleece stays intact. On the top side the free-standing film acts as a physical barrier which provides mechanical stability and swelling capabilities. All composites show no cytotoxicity and are biocompatible. The genipin crosslinked composites show an increase in mechanical stability, while having a slightly inferior effect on cell growth compared to the native system. Overall, the composites show a superior outcome over their individual components.

Conclusions
The proposed composite can increase cell adhesion and proliferation by the topographic cues of the electrospun fibers acting as a scaffold. At the same time, the modular design of the LbL free-standing films allows the tuning of physical properties like swelling or gaseous exchange as well as the introduction of antibacterial capabilities or a growth factor reservoir with controlled release. This makes the composite a promising starting point for the design of a novel wound dressing.

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keywords: wound healing, electrospinning, LbL, glycoaminoglycans, free-standing film

62825440248

ELECTROSPUN ORAL PATCHES FOR PAIN RELIEF OF DRY SOCKET

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Introduction
Wisdom tooth extraction is one of the most common surgeries carried out in the UK, and is notorious for causing post-operative complications. 1 in 6 patients experience a condition called alveolar osteitis "dry-socket" after wisdom tooth removal; a condition where the blood clot is lost and the extraction socket becomes painful. The condition is a serious problem and causes a financial burden to the NHS, with repeat hospital visits required. Treatment is aimed at managing the pain at the site of the extraction until the natural process of healing has occurred; this can take a number of weeks. Painkillers can be difficult to swallow due to soreness at the extraction site. Pain following wisdom tooth extraction and also dry-socket could be alleviated by the development of an oral patch to cover the wound post-surgery. We have recently developed a biodegradable, mucoadhesive oral patch that demonstrates long residence times in vivo (Santocildes-Romero et al., 2017). The patches are comprised of a two-layer electrospun polymer system; a highly bio-adhesive inner layer and an outer saliva-resistant, durable yet flexible protective layer. Here, we hypothesised that oral patches fabricated to contain the local anaesthetic, bupivacaine hydrochloride, and steroid, prednisolone, placed over a post-surgical socket could reduce post-operative pain, whilst also acting as a physical barrier to prevent blood clot dislodgment or bacterial infection.

Methodology
Patches were fabricated using uniaxial electrospinning, and the incorporation of bupivacaine and prednisolone investigated. Patches were characterised by examining fibre diameter, mass uniformity, thickness, pH, adhesion, swelling index, as well as measuring drug release.

Results
Fabricated oral patches were consistent in mass, fibre diameter and thickness. The patch was slightly alkaline when submerged in artificial saliva. Bupivacaine hydrochloride and prednisolone were released rapidly from the patch within the first 15 minutes, followed by gradual drug release over the next 45 minutes. In total the patches were able to release 0.3 mg of both bupivacaine and prednisolone over 60 minutes. An in vitro adhesion assay confirmed the ability of patches to attach to the oral mucosa for prolonged periods.

Conclusion
These data suggest that the mucoadhesive patches constitute a promising method for the prevention of post-operative pain and dry socket.

Santocildes-Romero, M., et al., ACS Applied Materials & Interfaces. 9(13), 11557-11567 (2017).

keywords: electrospinning, oral patches, drug delivery, dry socket.

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EVALUATION OF MASLINIC ACID AS A NOVEL PROMISING MOLECULE ABLE TO ENHANCE THE BIOFABRICATION PROTOCOLS OF TISSUE-ENGINEERED SKIN SUBSTITUTES

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Introduction: Severe skin lesions can be life-threatening conditions, especially in burnt patients. We recently described the UGRSKIN model of bioartificial human skin generated by tissue engineering as an ATMP1. Although preliminary clinical results are promising, the biofabrication procedure requires long periods of time due to the need of obtaining abundant keratinocyte cell populations from a single biopsy obtained from healthy skin areas. Reduction of the biofabrication time could contribute to an early treatment of burnt patients in critical risk. One of the methods used to increase the efficiency of keratinocyte culturing is the use of bioactive molecules able to induce cell proliferation. In this milieu, maslinic acid is a bioactive compound found in olive oil which has multiple benefits for health2. This molecule has been widely tested for different purposes, but its potential role in keratinocyte-based tissue engineering has not been determined to the date.

Methodology: In this study, we cultured human keratinocytes using a culture medium enriched with increasing concentrations of maslinic acid. Then, we analyzed cell viability and proliferation using several biochemical and metabolic methods.

Results: Results showed that maslinic acid was very safe for the cells, and concentrations associated to a decrease of cell viability were very high. However, the lowest concentrations resulted in increased cell viability and proliferation of human keratinocytes, with higher number of cells found at the concentrations of 1-5µg/mL.

Conclusions: These preliminary results point out the putative beneficial effects of maslinic acid to accelerate and improve the biofabrication process of human bioartificial skin.

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keywords: Maslinic acid, keratinocyte proliferation, skin, tissue engineering

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INVESTIGATING THE EFFECT OF BLOOD-IMPLANT INTERACTIONS ON THE RESPONSE OF SOFT TISSUE CELLS TO TITANIUM IMPLANTS

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Titanium-based dental implants have been highly optimized to enhance osseointegration, but little attention has been given to the soft tissue-implant interface, despite being a major contributor to long term implant stability. This is strongly linked to a lack of model systems that enable the reliable evaluation of soft tissue-implant interactions. Current in vitro platforms to assess these interactions are very simplistic, thus suffering from limited biological relevance and sensitivity to varying implant surface properties. The aim of this study was to investigate how blood-implant interactions affect downstream responses of different soft tissue cells to implants in vitro, thus taking into account not only the early events of blood coagulation upon implantation, but also the multicellular nature of soft tissue. For this, three surfaces (smooth and hydrophobic; rough and hydrophobic; rough and hydrophilic with nanostructures), which reflect a wide range of implant surface properties, were used to study blood-material and cell-material interactions, in the presence and absence of blood. While rough surfaces stimulated denser fibrin network formation compared to smooth surfaces, hydrophilicity accelerated the rate blood coagulation compared to hydrophobic surfaces. In the absence of blood, smooth surfaces supported enhanced attachment of human gingival fibroblasts and keratinocytes, while limited changes in gene expression and cytokine production were observed between surfaces. In the presence of blood, rough surfaces supported enhanced fibroblast attachment and stimulated a stronger anti-inflammatory response from macrophages than smooth surfaces, but only smooth surfaces were capable of supporting long-term keratinocyte attachment and formation of an epithelial layer. These findings indicate that surface properties govern blood-implant interactions, which in turn can significantly modulate the subsequent cell-implant interaction.

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keywords: Titanium implants, soft tissue integration, gingival fibroblasts, gingival keratinocytes, macrophages, human whole blood

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PROMOTION OF WOUND HEALING THROUGH CAZN-RELEASING NANOPARTICLES; IN VITRO STUDIES

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Introduction: Chronic wounds represent a major burden in human society, and their prevalence is expected to increase due to the upward trend of an aging population, incidence of diabetes, and obesity. In Europe, one out of five hospitalized patients suffers from a pressure ulcer. Moreover, the costs associated with its extensive care treatments are high; about 25 billion dollars are spent annually in the US1. These costs can be reduced by appropriate diagnosis and treatment. However, a device that enables fast-effective closure, low cost, and scalability is still missing.
Ions such as calcium (Ca2+) and zinc (Zn2+) are essential for skin homeostasis. It is well known that calcium regulates platelet aggregation and epidermal stratification. We have shown that calcium phosphate nanoparticles (NPs) stimulated wound healing both in vitro and in vivo 2,3. On the other hand, zinc deficiencies are associated with impaired wound healing and roughened skin. Zinc's antimicrobial properties have been recently suggested 4,5, making this ion promising for its application on wound dressings.
This work aims to develop an ion releasing platform based on nanocomposites for local and sustained calcium and zinc release at the wound site, to achieve wound closure and prevent microbial growth.

Methods: Submicrometric particles incorporating different amounts of Zn2+ and Ca2+ ions were developed. Ion release was analyzed by colorimetric methods. Particle size was determined by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Particle composition was studied using X-Ray Diffraction and Energy Dispersive X-Ray Spectroscopy (SEM-EDX). Direct and indirect particle toxicity was assessed in vitro in human dermal fibroblasts (hDFs) and human keratinocytes (hKCs) using the MTT assay. Cell migration was studied using the scratch wound assay. Collagen deposition and wound contraction were assessed by hydroxyproline assay and a fibroblasts-populated collagen lattice, respectively. Matrix metalloproteinases (MMP) production was studied by zymography.

Results and Discussion: Calcium and zinc were successfully incorporated into the particles with high reproducibility. Different compositions of Zn2+ and Ca2+ were combined successfully. Particles showed a rounded morphology, with submicronic sizes. Backscattering images show that zinc and calcium are homogeneously mixed. Ion release was sustained for up to 1 month (assay endpoint). Particles with lower Zn content showed better biocompatibility compared to higher ones. In vitro, particles promoted cell migration and collagen deposition. MMPs were not activated in the presence of nanoparticles.

Conclusion: Ion releasing platforms were successfully produced. Their composition, size, and ion release profiles as well as their in vitro performance indicate their potential use in wound healing therapies.

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keywords: Wound healing; Bioactive glass; ions

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PULSED ELECTRIC FIELDS AS PROMISING TOOL FOR TREATING SKIN FIBROSIS

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Introduction. Impairment of extracellular matrix (ECM) remodeling is observed in tumour microenvironment or fibrosis and results in excessive collagen production and/or its decreased degradation by metalloproteinases (MMPs). Due to its pivotal role in tissue architectures and functions, ECM components and the proteins that regulate ECM remodeling are thus promising therapeutic targets for human healthcare. Physical stimuli appear as attractive tools to remodel ECM owing to their local and space-time control effects 1. For that purpose, we assessed the potential of pulsed electric field technology, classically applied to drug and plasmid delivery, to modulate cutaneous cells behavior as well as remodel collagen at tissue scale.

Methodology. Two distinct calibrated electric protocols classically used for gene electrotransfer (GET) (10 square-wave pulses of 5 ms, 1 Hz) and electrochemotherapy (ECT) (8 square-wave pulses of 100 µs, 1 Hz) were applied in our studies without addition of any drugs.

Results. It appeared that dermal fibroblasts proliferation and migration properties were not affected when grown in monolayer, whatever the electric field intensity applied 2. We assessed the ECM remodeling after electroporation was applied onto a self-assembled tissue-engineered human dermal substitute model devoid of exogenous material, by examining genes modulation by transcriptomic and proteins synthesis, as well as MMPs activity. Fourier-Transform Infrared-Attenuated Total Reflectance and Differential Scanning Calorimetry were used as complementary tools to analysed collagen structure. We demonstrated that pulsed electric fields induced 1) a rapid modulation (4h after electrostimulation) of mRNA's genes composing the matrisome, particularly a down-regulation of pro-collagens and ECM maturation's enzymes; 2) a transient decrease in pro-collagens production and hydroxyproline tissue content within a week after electrostimulation; 3) a long-lasting ROS-dependent over-activation of MMPs and especially collagenase's family for at least 48h and 4) a down-regulation of TGF-β1, a key player in pathological fibrosis 3. Our first results also indicate that pro-angiogenic processes occur after pulsed electric field application.

Conclusion. Since electroporation is already used today in clinics (FDA and EMA approved), validated equipment (generator, electrodes, dosimetry) is available and doctors are used to this type of protocol. Our research therefore has a strong applicative potential. Taken together, our results open up realistic and relevant prospects for pulsed electric field technology as a local and effective treatment of skin abnormal ECM.

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keywords: collagens, extracellular matrix, migration, metalloproteinases (MMPs), electroporation

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THE ANTISEPTIC AND BIOCOMPATIBILITY PROPERTIES OF HYALURONAN CHLORAMIDE IN WOUND HEALING

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Introduction
Many refractory chronic wounds are infected. Hyaluronan chloramide (HA-Cl) is a novel derivative that combines pro-regenerative hyaluronan and antimicrobial chlorine. While the synthesis and stability of HA-Cl were described recently1, its oxidative activity, efficacy against bacteria, and safety in wounds remain to be evaluated.

Methodology
The rate of substrate oxidation mediated by HA-Cl was investigated by means of a tetramethylbenzidine (TMB) colorimetric assay. The amount of HA that resulted from HA-Cl oxidation was analyzed using LC-MS/MS chromatography. The antimicrobial effects of HA-Cl were evaluated in Staphylococcus aureus using both planktonic bacteria (a modified disk diffusion method and growth inhibition in liquid media), as well as bacterial biofilm (Calgary biofilm device). The biocompatibility of HA-Cl was investigated in mouse excisional 6-mm wounds. HA-Cl in the form of nanofibrous textile was applied onto freshly created wounds and reapplied every 2-3 days during redressings until harvesting the wounds on day 14. Wound healing was assessed by means of histology and qRT-PCR. The numbers and proportions of blood cells were assessed using a haemoanalyzer. In vivo observations were complemented by assessing human blood haemolysis induced by HA-Cl in vitro.

Results
HA-Cl exerted slower substrate (BSA) oxidation than that of NaOCl, and chloramine T (CAT) as assessed with the TMB assay. The oxidation rate of HA-Cl was markedly increased by NaI, which served as a catalyzer. The analysis of HA-Cl degradation products showed its conversion to pure hyaluronan. The slower substrate oxidation mediated by HA-Cl prolonged its antimicrobial effect in liquid culture. When 106 CFU/ml S. aureus was reinoculated into the same culture media, bacteria were able to grow only in the samples treated previously by NaOCl, while no growth was observed in the samples containing HA-Cl. HA-Cl efficiently eradicated bacterial biofilm. Aimed at investigating the safety of HA-Cl in vivo, the nanofibers containing HA-Cl were applied into mouse excisional wounds. Wound healing in the treated animals was normal when compared to controls in terms of granulation, inflammation, and epithelization ($p>0.05$). Blood profiles of the HA-Cl-treated animals did not deviate from those of untreated controls. In addition, HA-Cl, unlike NaOCl and CAT, did not cause haemolysis of blood in vitro ($p<0.05$).

Conclusions
HA-Cl is a safe, biocompatible, and efficient antiseptic, which is suitable for wound healing. It can be manufactured as sheets of nanofibers, which are conveniently applied topically. After HA-Cl is reduced in wounds, the resulting hyaluronan may further promote wound healing.

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keywords: Wound healing, hyaluronan chloramide, antiseptics, HA-Cl

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THE DEVELOPMENT OF GELATIN-BASED MICRONEEDLES PATCH WITH GALLIC ACID FOR THE PREVENTION OF KELOID SCARS

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Keloids are abnormal scar tissue characterized by excessive proliferation of fibroblast and overabundance of collagen. The keloids often occur on earlobes, shoulders, back of the neck and the chest. To date, the therapeutic treatment of hypertrophic scar includes corticosteroid injection, laser therapy, cryotherapy, radiotherapy, and surgical scar revision. However, none of the above methods colud completely cure healing. In this study, we developed a gallic acid-loaded microneedle patch with biocompatibility and biodegradability for inhibiting fibroblast proliferation as a preventive treatment of keloid. Gallic acid, which is found in most plants, has the inhibitory effect of fibroblast growth. Microneedle is a new and painless transdermal drug delivery. The microneedle with loaded drug can puncture stratum corneum, which improves the permeability and the efficiency of drug delivery. In this study, gelatin and hyaluronic acid, which have good biocompatibility and biodegradation, are used as the main materials of microneedle. Gelatin-based microneedles were prepared by crosslinking with various crosslinking agents. The results showed that the mechanical property of microneedles increased with increasing gelatin concentration. And the puncture performance of gelatin microneedles crosslinked with glutaraldehyde and EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) was 93%. However, it was observed that the puncture ratio of gelatin-based microneedles decreased with the increase of hyaluronic acid content. The in vitro degradation assay showed that gelatin-based microneedles could degrade rapidly within 30 min. We tested the antiproliferative effect of gallic acid loaded in the microneedle patches on fibroblasts. The proliferative capacity of the fibroblasts was significantly inhibited after 6 hours. The results demonstrated that the gelatin-based microneedle patches with gallic acid as drug developed in this study have a promising application in keloid treatment via transdermal delivery.

keywords: Keloid Scars, Gelatin, Microneedle, Gallic acid

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UNDERSTANDING THE MECHANISMS OF ACTION OF COLLAGEN-BASED DRESSINGS TO PROMOTE HEALING

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Introduction
Wound healing is a complex process that involves numerous cell types, cytokines, chemokines, growth factors and extracellular matrix (ECM) components, which work synergistically to achieve healing1,2. When the healing process fails to proceed through the four physiological phases, the wound is referred to as a chronic wound1,2,4,5. Chronic wounds are a significant global problem, causing patient morbidity and a substantial financial burden on health services worldwide. The rising prevalence of chronic wounds puts increasing pressure on global health services, calling for the development of therapies that can relieve both patients and healthcare systems of this economic and societal burden 6,7.
Collagen-based dressings are a large class of dressings that offer numerous beneficial properties for the treatment of recalcitrant wounds8-11. Commercially available collagen-based wound dressings differ in composition but present similar claims. There is a lack of comparative data to differentiate between these dressings and understand their mode of action.

Methodology
The dressings chosen for analysis were a control non-woven dressing (3M, Saint Paul, MN, US), 3M™ Promogran™ Protease Modulating Matrix and 3M™ Promogran Prisma™ Wound Balancing Matrix (3M, Saint Paul, MN, US), Puracol® (Medline Industries Inc., Northfield, IL, US), ColActive® Plus (Covalon Technologies Ltd., Mississauga, Ontario, Canada) and UrgoStart® (UrgoMedical, Chenôve, France), a synthetic dressing with similar claims to collagen-based dressings. Thirty-six diabetic (db/db) female mice received two full-thickness excisional wounds. Wounds were treated with a pre-moistened control or collagen dressings (12 wounds per group). Dressings were changed or re-applied after 3 days according to the products' IFU. After 7 days, macroscopic images were taken, and wounds harvested. Wounds were bisected and processed for histological and biochemical analysis.

Results
Wound area calculated from the macroscopic images for all wounds showed that 3 of the 4 collagen dressings and UrgoStart promote healing compared to the control ($p=<0.05$), achieving 60-70% closure within 7 days. Analysis of wound parameters from histological sections revealed greater re-epithelialisation compared to the control. Granulation tissue area, wound length and re-epithelialisation varied amongst the collagen dressings but no significant differences were observed.

Conclusions
Preliminary results show that collagen-based dressings promote healing of diabetic murine wounds to a better extent than control dressing. However, further in vitro analyses are needed to further elucidate these initial results. These results may help healthcare professionals with a greater understanding of how collagen-based dressing can modulate healing of chronic wounds.

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keywords: chronic ulcers, collagen, wound dressing, wound healing

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WOUND DRESSING BASED ON NANOFIBERS FROM HYALURONIC ACID AND HYALURONIC ACID DERIVATIVE

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Introduction
Using nanofibrous materials for wound healing is very promising yet still challenging. The dressing is usually a composite made of several layers, with a specific role to ensure proper moisture of the wound, breathability, low adhesiveness to the wound and prevention of the infection. One of the benefits that nanofibers have is the ability to easily incorporate antimicrobial drugs. In this work, we focused on the preparation of the wound dressing with two biologically active substances – hyaluronic acid, which plays an important role in wound healing processes, and a hyaluronic acid derivative with an antimicrobial effect. These biologically active ingredients are incorporated in the dressing in the form of a nanofibrous layer, which readily dissolves after contact with wound fluids.

Methodology
The presented wound dressing consisted of a nanofibrous layer based on antimicrobial hyaluronan chloramide (HA-Cl)1 and low molecular weight hyaluronic acid (LMW-HA). Nanofibers were prepared by electrospinning and laminated to a wound contact layer and an absorbent layer. The complete wound dressing was sterilized with ethylene oxide (EtOX). The morphology of nanofibers was studied by SEM. Residual solvents and residues after EtOX sterilization were determined by GC-MS. The amount of LMW HA was determined by HPLC and the amount of HA-Cl by iodometric titration. Permeation of moisture through the wound dressing was measured via a water vapor transmission test and the results were compared with a commercial product. The wound dressing was analyzed for the presence of bacterial endotoxins by the monocyte activation test (MAT). In vitro testing was used for the evaluation of the safety of the wound dressing. The effect of the wound dressing extracts on the viability of 3T3 fibroblasts and migration of HaCaT keratinocytes was assessed as well as the potential for skin irritation where the model of the human epidermis was used. The potential for skin sensitization of wound dressing extract was assessed by the dendritic cell activation test. The safety of the nanofiber layer was evaluated in vivo using a mouse model of an excisional acute wound.

Results
The large-format electrospun nanofibrous layers were laminated to the wound contact layer and the absorbent layer. The lamination did not affect the nanofibrous structure. The nanofiber structure and the content of the active ingredients did not change after sterilization and no EtOX residues and residual solvent were found. The uniform distribution of LMW HA and HA-Cl in nanofibrous mats was confirmed by HPLC and titration. The permeability of water vapor through the nanofiber wound dressing was similar to a commercial product. The safety of the wound dressing was evaluated by in vitro tests without any negative results. Furthermore, the tested nanofiber layer was well-tolerated when applied to acute mouse excisional wounds.

Conclusion

A new wound dressing with the active nanofibrous layer and homogenous content of bioactive components was produced using the semi-production electrospinning device. The results obtained so far have demonstrated the safety of the bioactive layer and promising results for future development.

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keywords: hyaluronic acid, hyaluronic acid chloramide, nanofibers, electrospinning

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WOUND HEALING EFFECTS OF AN ACELLULAR SKIN SUBSTITUTE IN THIRD DEGREE BURNS

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Burned skin wound treatment is one of the most expensive burdens on healthcare systems worldwide. The severity and extension of burn trauma could elevate the rate of hospitalization, and patients with burn wounds are especially vulnerable to infections. Burn injuries are very challenging to manage since it causes loss of the integrity of large portions of the skin, leading to major disability or even death. 1 In order to address limitations of conventional treatments and solve the problem of scarcity of donor grafts or immunological rejection with allografts, skin substitutes from tissue engineering emerged as an alternative.
In addition, these substitutes can prevent the growth of microorganisms and improves wound microenvironment, aids cellular behavior, reduces inflammation status and enhances tissue regeneration. 2,3 The aim of this research is to develop and evaluate the effects of the ARTSKin™ dressing in the early stages of treatment after burn. Acellular ARTSKin™ is a hydrogel-like biomaterial, consisting of 98% water and 2% natural polymeric nanofibers structurally similar to human collagen, mimicking the extracellular matrix, and thus providing a suitable microenvironment for human tissue manufacturing. In vitro studies prove the functional effects of the ARTSKin™ on cells, the non-toxicity of the biomaterial and its use as support for the cell cultivation in epidermal, dermal and/or dermoepidermal substitutes.
In vivo, ARTSkin was used as a skin substitute to overlie thermal burns induced on the dorsal skin of mice using a preheated brass template. Firstly, burned tissue was debrided and then overlaid with acellular ARTSkin. To create a full thickness burn wound, the brass block was applied at 100°C for 20 seconds, using a constant pressure. Multimodal analgesia was adopted for pain management. The administration regime of analgesia included preemptive or pre-procedural analgesic administration. 4 Histopathological investigation and microbiome of skin tissue of C57BL/6J mice showed almost accelerated wound closure compared with a standard control group. All the information obtained from this study indicated that the ARTSKin can be considered a promising skin substitute.

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keywords: full thickness burn; bacterial cellulose; preclinical research; wound healing.

PS57
Supramolecular synthetic scaffolds: from concept to design and application

20941835924

DESIGNING DYNAMIC AND PHOTO-RESPONSIVE DOUBLE NETWORK HYDROGELS FOR TISSUE ENGINEERING

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Recent works on dynamic hydrogels, using non-covalent and/or dynamic covalent linkages, have shown that dynamic and spatial-temporal complex materials are promising step-towards the recapitulation of extracellular matrix (ECM) functionality and structure 1-5. However, the uncontrollable network properties across timescales make these dynamic hydrogels insufficient for fully recapitulating the native ECM. In order to control the mechanical and biological cues provided to cells through the biomaterial at each stage, we investigated the combination of two networks: one dynamic covalent cross-linked, and the other a photo-responsive network. The second network based on light-responsive linkages could enable reversible control of cross-linking degree with spatiotemporal resolution. Here, we first synthesized photo-responsive polymers based on polyethylene glycol with a coumarin derivate as an end group. After optimizing the synthetic protocol, the coupling-cleavage transition of 4-arm-polyethylene glycol coumarin (4PC) and 2-arm-polyethylene glycol coumarin (2PC) was determined by UV-Vis spectroscopy. The dimerization of coumarin takes place by irradiation above 300 nm, while the cleavage of the dimers is below 300 nm. Once the photo-responsive polymers were characterized, double network (DN) hydrogels were developed by combining a dynamic and a static photo-responsive cross-linked network. We investigated the combination of oxidized alginate cross-linked by Schiff-base reactions (OA, dynamic network) and photo-cross-linkable polymers (2PC and 4PC, photo-responsive network). The DN hydrogels showed self-healing properties and their mechanics can be modulated by UV irradiation. Rheological characterization was carried out to further investigate the reversibility of the hydrogel properties by UV irradiation. These biomaterials are promising for remotely providing to cell mechanical and biological cues and, hence, modulating cell response.

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keywords: double networks, hydrogels, photo-responsive

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NEAR-INFRARED LIGHT-TRIGGERED CORE-SHELL UPCONVERSION NANOPARTICLES FOR THERANOTICS

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Combining upconversion nanoparticles (UCNPs) and UV-sensitive polymers to form a drug delivery system (DDS) is a promising strategy to circumvent drawbacks of direct UV excitation in clinical applications. This study tuned up core-shell UCNPs with shell thickness of 6 nm and emission wavelength falling in the ultraviolet region at 350 nm under near-infrared (NIR) light irradiation at 980 nm. An amphiphilic block copolymer with UV-responsive o-nitrobenzyl ester (ONB) next to glutathione (GSH)-responsive disulfide linkage was synthesized and formulated into polymersome. Core-shell UCNPs and doxorubicin (DOX) were simultaneously encapsulated into the polymersome during double emulsion for theranotics. The combination of NIR light-inducing photolysis of the ONB linkage and GSH cleaving the disulfide linkage enhanced DOX release for chemotherapy. This intriguing polymersome of well-defined structures responsive to NIR light and reducing agents offers potential for DDS applications.

keywords: polymersome, dual-stimuli, near infrared (NIR) light, upconversion nanoparticles, drug delivery system

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NEW STRATEGIES TO IMPROVE STABILITY OF GUANOSINE-BASED SUPRAMOLECULAR HYDROGELS FOR SOFT TISSUE REGENERATION

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Introduction
Nowadays, soft tissue damage are typically replaced by grafts from other body parts. However, tissue grafting can be problematic due to the need for several surgical procedures or lack of sufficient tissue material. Recently, hydrogels have emerge as an alternative treatment for tissue regeneration. Specifically guanosine-based hydrogels show unique self-assembly properties resulting in hydrogels with high water content and nanofibrilar structure mimicking the extracellular matrix. Four planar guanosine-quartet can lead to 3D nanofibrous G-quadruplex (G4) structures by π-π stacking interactions between aromatic purine rings that under certain conditions, are able to encapsulate enough water to produce G4 hydrogels. Combined with 3D printing technology, they could provide a highly hydrated and well-defined 3D environment optimal for cell survival and differentiation. However, hydrogels currently lack lifetime stability, biological activity, and printability, limiting their use and applicability. We hypothesized that using more complex boronic acid derivatives such as phenylboronic acid, 2-formilphenylboronic acid, and 2-naphtylboronic acid, in addition to boric acid, would further enhance G4 and thus hydrogel stability.

Experimental Methods
Guanosine, boronic acid derivatives, and potassium hydroxide (20-120 mM) were mixed at 80 °C for 15 minutes and cooled down for gelation. Inversion test was performed to select compositions capable of gel formation. Next, printability properties were determined by a semi-quantitative filament fusion and collapse test. The best hydrogels were then evaluated by scanning electron microscopy and dynamic step-strain sweep and peak-hold rheology assays. Additionally, hydrogel pH, degradability in the complete medium, and nutrient diffusion (FITC-Dextran) were determined. Viability of SaOS-2 cells seeded onto the printed hydrogels was also assessed using confocal laser scanning microscopy and live-cell mapping.

Results
A total of 76 hydrogel compositions passed the initial inversion test, of which ~1/3 showed

suitable properties in the filament collapse and fusion test. We then selected the best composition for each boronic acid derivative for a comprehensive analysis. SEM analysis revealed nanofibrillar structures in the hydrogel networks of all four boronic acid derivatives, evident of successful G4-quadruplex formation, and rheological testing confirmed good thixotropic properties. Importantly, while all tested hydrogels showed a pH between 7.4 and 8.3, only hydrogels obtained with boric acid were stable for up to 7 days. Furthermore, successful diffusion of FITC-Dextran molecules (70, 500 and 2000 kDa) into the hydrogel was observed, indicating that nutrients of various sizes may be transported through the scaffold, and finally, a cell viability of ~80% after 24 hours was determined for SaOS-2 cells seeded onto the printed hydrogels.

Conclusions
We developed a novel printable hydrogel based on guanosine and different boronic acids. Only boric acid hydrogel showed good printability and thixotropic properties and was stable in medium for up to 7 days, while no cytotoxicity was observed. Thus, this hydrogel formulation represents an excellent candidate for initial steps of angiogenesis or as potential antibacterial biomaterial for biomedical applications.

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keywords: Supramolecular hydrogels, G-quadruplex, Guanosine, 3D printing

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SELF-ASSEMBLING PEPTIDE GELS FOR ARTICULAR PATELLA CARTILAGE REPAIR

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INTRODUCTION
Loss of cartilage glycosaminoglycans (GAGs) leads to reduced biomechanical function [1]. This change is seen in the early stages of osteoarthritis (OA) and in other joint disorders such as Chondromalacia patella (CP), where softening of patella cartilage is associated pain. There is a clinical need for early intervention treatments to restore cartilage function and delay the progression of cartilage degeneration. Delivery of self-assembling peptide (SAP) hydrogels combined with chondroitin sulfate (CS) into damaged cartilage may restore the GAG content and biomechanical function, specifically patella and femoral cartilage [2] [3].
The aim of this study was to develop an in vitro GAG-depleted patella model and assess the biomechanical effects following treatment with a SAP:CS self-assembling hydrogel.

METHODOLOGY
Model: 4-6 month old porcine patellae were harvested and washed with 0.1% (w/v) SDS to remove GAGs from the cartilage. Histological and biochemical analysis was carried out to assess GAG removal and any changes to the tissue architecture (n=6).
SAP:CS treatment: Indentation testing (n=6 per group) was performed on native, GAG depleted patellae, and a SAP:CS treated group. SAP:CS (~ 6mM SAP and 10 mg CS in Ringers) was injected into a 10 mm2 area of cartilage through a 30 G needle.

RESULTS
The GAG depletion process removed 56±12% of the sulphated GAGs within porcine patella cartilage (mean ± 95% C.I.). Histological analysis of the GAG depleted samples showed GAG loss with the remaining architecture unaffected.
A significant increase in percentage deformation was seen in the GAG depleted group compared to native cartilage (n=6, $p<0.001$). Treated samples showed significant reduction in percentage deformation compared to the depleted group (n=6, $p<0.05$) and no significant difference to native cartilage (Figure 1).

CONCLUSIONS
The ~50% reduction of GAGs represented a moderate osteoarthritic cartilage model and did not cause observable changes in the tissue architecture or collagen orientation. GAG loss resulted in a significant reduction in cartilage stiffness which was successfully restored by treatment with SAP:CS. Future work is underway to investigate the biotribological effects of SAP:CS through the use of a six-axis natural joint simulator. SAP:CS has potential to be an effective treatment for CP and early OA.

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keywords: Self-assembling peptides, cartilage, biomaterials, biomechanics, musculoskeletal

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SELF-ASSEMBLING PEPTIDE HYDROGELS FOR COLORECTAL ORGANOID CULTURE

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Introduction
Organoids have the potential to revolutionise drug discovery and development, providing an in vitro platform to determine drug efficacy and toxicity while reducing the need for animal testing. However, the wider adoption of organoids is held back by reliance on animal derived matrices for the production and culture of organoids. Animal derived materials are not only unethical but also lead to high batch-to-batch variability and xenogenic contamination and are therefore unsuitable for preclinical processes. Currently the field lacks a suitable, widely adopted, synthetic 3D cell culture environment for organoid culture and wider adoption into the drug development pipeline.

The aim of this project is to develop a new, fully synthetic (non-animal derived) 3D matrix which is optimised for the growth of colorectal organoids and can be applied in the future for industrial-scale organoid production and high throughput applications for several preclinical studies.

Methodology
PeptiGels® were produced by Manchester BIOGEL Ltd. The stiffness was measured using oscillatory rheology. The suitability for organoid-relevant cell culture was determined by qualitatively analysing the structural integrity of 50µL domes of different PeptiGels® over a course of 21 days under normal cell culture conditions. Colorectal organoids provided by Cellesce Ltd were cultured for up to 21 days, viability was assessed by LIVE/DEAD assay. DIC light microscopy was used to obtain images of organoids during their culture. Using ImageJ analysis, the cross-sectional area of organoids was generated.

Results
We assessed PeptiGels® Gamma 2 included within the hydrogels were 4 different functional biomimetic peptide motifs -GFOGER (Collagen), -RGD (fibronectin), -IKVAV (Laminin) and -YIGSR (Laminin). All combinations of the hydrogels had stiffnesses matching physiological tissue stiffness (between 0.5 and 3 kPa). Creating hydrogels using the 'dome method' (a gold-standard method for organoid culture), the acellular hydrogels were stable for up to 21 days in culture. Next, human primary colorectal organoids from Cellesce Ltd were cultured within the hydrogels. The ISO68 organoid line formed viable organoids of an equivalent size and shape to organoids grown in Matrigel, the organoids increased in cross-sectional area over the course of 14 days. Finally, the inclusion of functional motifs -IKVAV (Laminin) and -GFOGER (Collagen) into Gamma2 PeptiGels® showed enhanced organoid growth when compared to Gamma 2, Matrigel, Gamma 2-YIGSR and Gamma2-RGD.

Conclusion
This initial work highlights the potential for PeptiGel® Gamma2 for use in colorectal organoid manufacture and culture. Future work will focus on expanding the potential of PeptiGels® for use with other organoid types.

keywords: organoids, hydrogels, biomaterials, 3d cultures, stem cells

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SYNTHESIS, FABRICATION, AND CHARACTERIZATION OF A BIO-INSPIRED, TISSUE-ADHESIVE CARDIAC PATCH FOR TISSUE ENGINEERING APPLICATIONS

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Introduction
As one of the most outstanding technology, cardiac patches hold the potential to restore cardiac function clinically. Many biomaterials used to fabricate cardiac patches have emerged during the last decade. Synthetic polymers can be tailored on a molecular level to fit any requirement that should be met to function as an integral part of a beating heart.
A facile method was found to incorporate a mussel inspired adhesive moiety into polyurethanes to develop a tissue engineered electrospun cardiac patch.

Methodology
The polyurethanes were synthetized through a step growth polymerization based on 1,4-butanediol (BDI) as hard segment, triblock copolymers PCL-PEG-PCL as soft segments, and lysine-dopamine as chain extender.
The triblock copolymers PCL-PEG-PCL were synthetized by Ring Opening Polymerization, setting two molar ratios between ε-caprolactone and Polyethylenglicole equal to 30 and 50 respectively.
Lysine-dopamine LDA was synthetized from L-Lysine and dopamine-HCl as reported in literature.

Products were characterized by FTIR, 1HNMR, SEC, DSC and UV-Vis confirming the success of the synthesis reactions.

To characterize intrinsic properties of the polymer like cytocompatibility, adhesiveness after melting and degradation rate, the polyurethanes were processed by film casting. The synthetized polyurethanes were then dispersed in HFIP and processed through electrospinning to obtain a three-dimensional scaffold that mimic as closely as possible the structure of healthy native myocardium. SEM was adopted as investigation method to study the morphology of the fibers.

Results
Two triblock copolymers were synthetized and used to obtain different polyurethanes composition and properties. Lysine was used as chain extender alone or linked with dopamine to create a new type of mussel mimetic polyurethanes. The successful combination of the unique mussel-inspired adhesive moiety with a tunable polyurethane structure can increase cells adhesion and proliferation and tissue adhesion.

The DSC analysis showed two different melting temperature inside the polyurethane. The first one at 38°C is correlated to the PEG block ed the other at 56°C is due to the PCL. This result, combined with the observed adhesiveness after melting, could allow the safely attachment of the scaffold to the heart's wall when the temperature is increased locally.

The degradation test underlines the remarkable hydrolytic resistance of the synthetized PU which is a fundamental design requirement for cardiac patches to maintain structure and

function over time. Its properties and degradation profile could be controlled during the manufacturing process to provide the best outcomes for ECM synthesis and tissue regeneration.

Fiber deposition was macroscopically smooth and homogeneous along the metal rod, without any spikes. An almost linear relation between the thickness of the electrospun layer and the deposition time was observed.

Conclusions
The aim of this work is to develop a fully integrated cardiac patch begins with materials that support biological activity while minimizing the risk of additional injury for the patient, caused by the conventional stitching methods, and withstand the dynamic forces of the heart.
Conductive polymers may integrate more successfully since they are able to participate in the pumping of the heart. Indeed, we are currently working on electroactive components integration inside the fibrous scaffold.

keywords: Biomaterials, cardiovascular, polyurethanes, bio-fabrication, tissue engineering

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TUNEABLE SYNTHETIC PEPTIDE HYDROGELS TO PROVIDE PHYSIOLOGICALLY AND CLINICALLY RELEVANT IN VITRO 3D CULTURES

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Introduction
3D cell culture is an increasingly reliable method to mimic the in vivo environment in vitro and offers a robust platform for several investigations ranging from disease modelling, regenerative medicine to drug discovery and development. In addition, having more physiologically relevant 3D models of both healthy and diseased tissues will allow the better understanding of the key cellular processes and a more reliably development of safer and more effective therapies. Advancement in preclinical in vitro 3D models such as spheroids and organoids has made use of biomaterials to mimic the complex in vivo environment; however, some widely used biomaterials have limitations as they are animal derived and lack of tuneability to faithfully mimic the in vivo counterpart. Recent advances in the use of tuneable synthetic peptide hydrogels, such as PeptiGels®, have shown potential to overcome these limitations by better simulating tissue microenvironments for enhanced research, allowing the generation of more physiologically and clinically relevant data.

Methodology
Tuneable PeptiGels® were optimised (matrix stiffness and functionality) for the growth and culture of mesenchymal stem cells, pancreatic (Suit-2) and breast cancer cells (MCF-7, MDA-MB-231). The cultured cells were characterised by analysing their viability (live/dead assay), integrity and homogeneity using imaging techniques (brightfield and immunofluorescence). Mesenchymal stem cells cultured in PeptiGels® were differentiated towards osteogenic lineages. The differentiated cells were phenotyped (Immunocytochemistry and confocal imaging) for key bone markers (collagen-I, osteocalcin, alkaline phosphatase). Pancreatic and breast cancer cells cultured in PeptiGels® were examined for key tumour features (hypoxia and invasion) and drug penetration and efficacy.

Results
Findings here demonstrated the use of tuneable PeptiGels® for the growth of cells to develop complex models such as organoids, tumour models, and their applications more broadly within regenerative medicine and drug discovery. PeptiGels® provided a 3-dimensional platform to support the growth and differentiation of mesenchymal stem cells into osteoblasts. The differentiated cells remain viable, proliferated throughout the duration of culture and displayed key functional capabilities by the deposition of key proteins (Col-1), osteocalcin, and alkaline phosphatase in addition to the presence of mineralization within the hydrogel, indicating that PeptiGels® can support the differentiation of stem cells into lineages of interest and has potential for tissue regeneration in different disease contexts.

The use of PeptiGels® also generated physiologically relevant in vitro 3D breast and pancreatic cancer models to replicate healthy and diseased human tissues. PeptiGels® were fine-tuned to recapitulate the tumour microenvironment and study cancer-specific biology such as pH modulation, hypoxia and changes in the mechanical properties involved in cancer cell activation and survival. PeptiGel®-based breast cancer 3D models allowed drug penetration and were

resistant to Taximofen treatment when compared to scaffold-free 2D models, potentially offering a suitable physiologically relevant model for advanced drug discovery and development.

Conclusion
In conclusion, these tuneable peptide hydrogels - PeptiGels® are non-toxic, biocompatible, biodegradable and are tuneable to simulate different tissue microenvironments to provide physiologically and clinically relevant data.

keywords: 3D culture models, Synthetic peptide hydrogels, in vitro tissue models, disease modelling, synthetic scaffolds

PS59
The role of multifunctional nanomaterials in new tissue regeneration strategies

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DEVELOPMENT OF NANOSYSTEMS FOR DELIVERY OF PRO-REGENERATIVE PROTEIN TSG-6, WITH NEUROLOGICAL APPLICATIONS

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Introduction. With limited treatment options for acute brain injuries like stroke or traumatic brain injury, recent research has focused on the development of immune-modulating and pro-regenerative therapies for brain diseases. TSG-6 (tumor necrosis factor-alpha stimulated protein 6) is a multifunctional protein with attractive therapeutic potential for neurological applications. As for numerous therapeutic proteins, the in vivo efficacy of TSG-6 is limited by short half-life and reduced availability at the target site due to endogenous degradation. This limitation can be overcome by using delivery systems. Here, we describe the development and in vitro efficacy of nanosized particles for TSG-6 delivery specifically designed for use in the nervous system.

Methodology. Recombinant human (rh) TSG-6 was encapsulated into chitosan-hyaluronic acid (HA) nanoparticles and liposomes before further nanoparticle characterisation by transmission electron microscopy (TEM) and dynamic light scattering (DLS), and assessment of encapsulation efficiency. To confirm that the delivery systems improve the anti-inflammatory effect of rhTSG-6, mouse microglial BV2 cells and human CMEC/D3 brain endothelial cells were stimulated using inflammatory lipopolysaccharide (LPS) or interleukin-1 beta (IL-1β) and treated with rhTSG-6 containing nanoparticles. Cell viability was assessed by resazurin-based AlamarBlue assay and levels of inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were measured by ELISA.

Results. Synthesized HA-based nanoparticles and liposomes were within the nanometric range, as confirmed by TEM and DLS analysis, and have allowed efficient rhTSG-6 encapsulation. Nanoparticles did not affect cell viability and nanoparticle-delivered rhTSG-6 has successfully decreased IL-6 and TNF-α levels following inflammatory stimulation.

Conclusions. By exploiting the natural affinity of TSG-6 protein for HA, we have synthesized nanoparticles for rhTSG-6 delivery which successfully decrease inflammation in brain cells in vitro. Short-term future work will assess the biodistribution profile in vivo and enhancement of anti-inflammatory effect in a mouse model of systemic inflammation. Long-term future work will test nanoparticle-delivered rhTSG-6 as a therapeutic agent for brain tissue repair and functional recovery following ischaemic stroke.

keywords: delivery systems, liposomes, nanoparticles, drug delivery

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IMMUNE COMPATIBILITY OF 2D BISMUTHENE NANOSHEETS FOR FUTURE COMBINED MAGNETIC HYPERTHERMIA AND PHOTOTHERMAL THERAPY

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Introduction: 2D pnictogens, including bismuthene, have recently emerged as new members of the ever-increasing 2D family thanks to their exceptional electronic, topological, thermoelectronic, and optical properties.
Here we present a 2D bismuthene synthesized from the 3D bulk bismuth involving a surfactant-assisted chemical reduction method to improve its magnetic properties specifically.
Envisaging the future biomedical applications and considering the central importance of this material as a possible theranostic agent for photothermal and photodynamic therapy of cancer, the bio- and immune-compatibility of 2D bismuthene remains a fundamental step of its clinical translation.

Methods: Here we applied our experience gained on 2D nanomaterials in this context1–3 to evaluate the impact of 2D bismuthene on whole blood and immune cells by means of flow cytometry, complete blood counts, and multiplex cytokine analysis.

Results: 2D bismuthene immune profiling on whole blood and functional studies with human peripheral blood mononuclear cells showed the excellent bio and immune compatibility of the material, as well as the ability to modulate the immune response with anti-inflammatory properties. Interestingly, the bismuthene did not boost the expression of activation markers on T cells and monocytes, but it actually reduced them. A significant decrease in the expression levels of CD69 and CD25 was observed for T cells and monocytes, giving proof of a powerful CD25 and CD69 modulation on both immune cell types. The cytokines found expressed, such as IL4, IL5, IL6, TNFα, and IFNγ, resulted in a non-significant difference with respect to the

untreated sample.

Conclusions: Taken together, these results describe bismuthene as a highly biocompatible 2D nanomaterial able to modulate the immune response with anti-inflammatory properties, a crucial aspect for the development of anti-inflammatory drugs and cancer nanotherapeutics4–6. As the link between cancer and inflammation is investigated, nanoscientists can engineer targeted nanomaterials to efficiently combat the tumor-associated inflammation and cancer carcinogenesis, dissemination, and metastasis.

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keywords: cancer therapy, bismuthene, magnetic field, 2D materials, immune system

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NOVEL HYDROGEL-BASED BIOPOLYMERIC FILMS FOR LOCAL TMZ DELIVERY

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Glioblastoma (GBM) is the most common (60-70% of primary brain tumours) and the most malignant of the glial tumours. Although current therapies remain palliative, they have been proved to prolong overall survival. The effectiveness of temozolomide (TMZ) chemotherapy is limited by the serious systemic and dose-related side effects. Therefore, the primary goal of the presented studies was the design, fabrication, and preliminary characterization of the biopolymeric-based materials serving as a system for the local delivery of TMZ. The only clinically approved to date implantable therapeutic formulation for GBM therapy is Gliadel which releases carmustine, however, there are no analogous products containing TMZ available. The proposed approach can increase TMZ concentration at the desired site simultaneously reducing adverse systemic complications.

The major constituent of the obtained material is a lyophilized form of a hydrogel, crosslinked with a genipin matrix based on three biopolymers: collagen, lysine-modified hyaluronic acid (HAmod), and chitosan. Ionically gelled with tripolyphosphate chitosan particles loaded with TMZ were incorporated into the polymeric sol, which upon formation of covalent bonds formed biomaterial. For such a system several experiments were conducted like- swelling, degradation and the TMZ release study. To prove the possibility of a tuneable TMZ release profile two modifications of the presented material were tested. The former concerns the additional functionalization of chitosan particles with HAmod, whereas the second is related to attaching the TMZ molecules to the chitosan chains using EDC/NHS chemistry, which demands the transformation of TMZ into its derivative, TMZ-COOH. Fabricated carriers were characterized in terms of their morphology, size, and stability by means of the scanning electron microscope (SEM), dynamic light scattering technique, and zeta potential measurements, respectively. The encapsulation efficiency was estimated based on UV-Vis spectra. The products synthesized within the second modification were characterized by FTIR and NMR spectroscopies.

SEM microphotographs revealed that both types of particles are embedded into the porous polymeric matrix. An increase in the carriers' size from approx. 100nm for chitosan ones to over 350nm for those modified with HAmod along with changes in their potential zeta values confirmed the desired functionalization. UV-Vis spectra enabled the verification of the TMZ content in the carriers. It was demonstrated that by playing with material composition and applying the lyophilization process the swelling ratio of the obtained products can be tuneable to potentially reduce the "mass effect" in the brain. The presence of the drug in the whole system was confirmed, however, TMZ encapsulated in the chitosan particles placed into the matrix exhibited the burst release. NMR and FTIR analyses identified the product of TMZ transformation as TMZ-COOH and verified its further immobilization to the chitosan.

Our findings suggest that the presented herein lyophilized hydrogel-based material could pose a reasonable starting point for the development of a novel local delivery system of TMZ, which has tuneable physicochemical characteristics. It is believed the proposed two modifications will help to achieve a more favourable, controlled release profile of TMZ.

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keywords: Hydrogels, Biopolymers, Temozolomide, Drug delivery

20941859208

PALLADIUM NANOPARTICLES IN HYDROGELS FOR CATALYTIC PRODRUG ACTIVATION AND CONTROLLED DRUG RELEASE

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Introduction:
The ongoing search for novel drug delivery to a target site has led to increased interest in using bioorthogonal organometallic (BOOM) chemistry for localised prodrug activation. Anticancer prodrugs containing a palladium (Pd)-cleavable propargyl protecting group have been developed, necessitating a suitable method to deliver the palladium nanoparticle (PdNP) catalyst to a desired location. PdNP-immobilisation within a non-degradable, biocompatible polymer matrix enables implantation of the catalyst, e.g. intratumourally. Systemic administration of a harmless Pd-sensitive prodrug results in the localised generation of therapeutic concentrations of drug.
PdNP-functionalised poly(ethylene glycol) (PEG) microbeads were prepared, and immobilised within a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel matrix. Catalytic activity of the materials was examined using a Pd-labile fluorogenic prodye. Absorption and release of the dye Resorufin (Res) by pHEMA was also investigated.

Methodology:
ChemMatrix® aminomethyl-PEG microbeads were functionalised with PdNPs by reduction of coordinated palladium(II) acetate with hydrazine monohydrate. PdNPs were physically entrapped at the surface by coupling surface amino groups to glutaric acid
Cross-linking of HEMA and ethylene glycol dimethacrylate (EGDMA), containing sheared gellan gum (GG) ± PdPEG microbeads, was initiated with ammonium persulfate/ tetramethylethylenediamine (APS/TEMED). Hydrogel films of varying thicknesses were cut to discs with a biopsy punch, yielding a cross-linked pHEMA±PdPEG disc.
Catalytic efficiency of free PdPEG and pHEMA-PdPEG were evaluated by incubation with Pd-sensitive probe, propargylated Resorufin (Pro-Res), and measuring the increase in fluorescence intensity.
Diffusion and absorption were measured by monitoring the decrease in fluorescence of a solution of Res containing a pHEMA disc over time, until equilibrium is achieved.

Results:
PdPEG-loaded pHEMA was prepared by polymerisation of monomer and bead mixture in the presence of gellan gum. Inclusion of gellan gum within the mixture aids suspension of the beads during the cross-linking process, facilitating homogeneous dispersion of PdPEG microbeads throughout the network. Microbead immobilisation was successful with no leaching of beads occurring after several months.
The catalytic ability of PdNP-functionalised PEG microbeads (PdPEG), in solution or immobilised in a pHEMA matrix, was demonstrated by the fluorogenic depropargylation of Pro-Res. PdPEG beads achieved >75% conversion after 24 hours in biologically relevant conditions. The pHEMA immobilised-PdPEG achieved similar conversions, under the same conditions, after 72 hours due to the rate limiting diffusion step.
Decrease in fluorescence intensity, proportional to concentration, of a solution of Res containing a pHEMA disc quantified dye uptake. The dye exhibited a preference for pHEMA over the aqueous solution of more than two orders of magnitude. Further understanding and

exploitation of this “depot effect” will aid design of a hydrogel implant which activates prodrugs and captures drug molecules to control their release.

Conclusions:
Entrapment of PdNPs within polymers, with retention of catalytic ability, was exhibited. Synthesised materials depropargylated fluorogenic Pro-Res, a model compound for Pd-sensitive prodrugs.
Immobilisation of PdPEG beads within pHEMA slowed prodye conversion, due to the rate-limitation of diffusion. Res exhibited preference for the pHEMA matrix over aqueous solution, likely due to hydrophobicity. Future work seeks to understand this behaviour, and modulate hydrogel implant properties to control this diffusion in/out of pHEMA.

keywords: palladium nanoparticles, pHEMA, drug delivery

31412743086

SMART-MATERIALS BASED DYNAMICALLY ACTIVATED MICROENVIRONMENTS FOR TISSUE ENGINEERING

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Smart and multifunctional materials are increasingly being used in tissue engineering and regenerative medicine in order to dynamically provide specific clues to the cells. In particular, the use of magneto- and electroactive polymers that deliver electrical signals to the cells upon magnetic or mechanical solicitation, respectively, open new scientific and technological opportunities, allowing the development of suitable biomimetic microenvironments for tissue regeneration. In fact, electrical and electromechanical clues are among the most relevant ones in determining tissue functionality in tissues such as muscle and bone, among others, indicating their requirement for proper tissue regeneration.
This talk reports on the electroactive and magnetoelectric materials used for tissue engineering applications. The most used materials and morphologies are reported, together with novel bioreactor designs allowing to take full advantage of those materials. The main achievements, challenges and future needs will be presented and discussed.

Acknowledgments
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keywords: electroactive microenvironments, smart materials, tissue regeneration

PS61
Tissue Engineering in Microgravity for Health in Space and on Earth

83767206447

DO WOUNDS HEAL IN SPACE? - MATRIX REMODELING AND FIBROBLAST DIFFERENTIATION IN SIMULATED MICROGRAVITY

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Exposure to microgravity affects astronauts' health in adverse ways. However, less is known about the extent to which fibroblast differentiation during the wound healing process is affected by the lack of gravity. One of the key steps of this process is the differentiation of fibroblasts into myofibroblasts, which contribute functionally through extracellular matrix production and remodeling. In this work, we utilized collagen-based three-dimensional (3D) matrices to mimic interstitial tissue and studied fibroblast differentiation under simulated microgravity (sμG). Our results demonstrated that alpha-smooth muscle actin (αSMA) expression and translocation of Smad2/3 into the cell nucleus were reduced upon exposure to sμG compared to the 1g control, which suggests the impairment of fibroblast differentiation under sμG. Moreover, matrix remodeling and production were decreased under sμG, which is in line with the impaired fibroblast differentiation. We further investigated changes on a transcriptomic level using RNA sequencing. The results demonstrated that sμG has less effect on fibroblast transcriptomes, while sμG triggers changes in the transcriptome of myofibroblasts. Several genes and biological pathways found through transcriptome analysis have previously been reported to impair fibroblast differentiation. Overall, our data indicated that fibroblast differentiation, as well as matrix production and remodeling, are impaired in 3D cultur

keywords: 3D cell culture, fibroblast differentiation, matrix remodeling, microgravity, tissue repair

94238144199

SIMULATED MICROGRAVITY MODIFICATIONS IN MUSCULOSKELETAL CELLS

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Introduction
Gravity is one of the four fundamental forces that govern the universe and that we experience in daily life without realizing it.
The absence of mechano-stimulation caused by the lack or reduced presence of gravity (microgravity) has been widely studied since the beginning of the space age. This condition creates various cascade reactions in human, resulting in a profound remodeling and adaptation of organs and tissues in the organism. In particular, osteopenia and sarcopenia are two of the first changes observed after space flights that impact deeply astronaut wellness.

We are studying the effects of microgravity in vitro through the Random Positioning Machine (RPM), a tool capable of simulating it. This instrument has a plane that moves on two rotation axes at a random speed and direction leading to the nullification, averaged over time, of the gravity vector. The cells we are studying are C2C12 and MLO-Y4 which are respectively myoblast and murine osteocyte cell lines.

Methodology & results
In the C2C12 cell line we started to study the expression of differentiation genes such as MyoD, MyoG and the different isoforms of MHC at different times of culture (1, 3, 5 and 7 days) in ground control and on RPM. Through Real time PCR analysis of early and late differentiation marker genes it has been seen that this condition leads to a slowing of differentiation process which becomes statistically significant at 5 days in the case of MHC 2 alpha mRNA expression ($P \leq 0.05$).

In the case of the osteocyte cell line MLO-Y4, we noticed cell behavior changes after 5 days in RPM. In particular, connexin 43, a protein that forms gap junctions between cells with key functions in signal transduction and in response to hormonal and mechanical stimuli was downregulated in RPM ($P \leq 0.0001$). Furthermore, genes linked to cellular senescence such as P53 (tumor suppressor), P21 and P16 were found to be much reduced in simulated microgravity (P53 = $P \leq 0.01$, P21 = $P \leq 0.0001$). Finally, the quantification of BAX mRNA, factor that regulates cell death, showed a reduced expression in RPM statistically significant ($P \leq 0.001$).

Conclusion
Further experiments will be needed to fully understand the complex connection that links the expression of these genes and microgravity exposure.

keywords: Osteocyte Microgravity RPM Myoblast

PS62
Tissue regeneration by integration of bioinspired materials

20941804266

4D BIOFABRICATION OF FIBROUS SELF-FOLDING MATERIALS

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The field of biofabrication is continually growing due to the increasing life expectancy of humans. The most promising technique in this field is 3D bioprinting, nevertheless, there are still several limitations like low resolution, the challenging printing of hollow tubular structures with low wall thickness due to high shear rates, and low cell alignment. 4D biofabrication is a technique where the fourth dimension is introduced by using smart materials, that are capable to undergo shape transformation after the addition of certain stimuli. The fourth dimension offers advantages such as hollow structure fabrication, various shape formations, and preservation of 2D patterns in the 3D structure. Based on the design using 4D biofabrication it is possible to fabricate highly complex structures with specific/ uniaxial cell alignment that resembles blood vessels, muscle tissues, lungs, etc.
Electrospinning allows the formation of highly aligned fibers in the sub-micron/micron range, that mimics natural fibers in the extracellular matrix. Fibrous electrospun mono-, bi-, and multi-layers by addition of stimuli like temperature and/or aqueous media, can transform into a tubular structure.
We have designed various fibrous shape-morphing systems that can be used as autografts for nerve, blood vessel, and muscle tissue regeneration. The extra high porosity of fibrous shape-morphing materials not only allows fast actuation rates (10s) but as well supports good nutrition and waste product exchange [1-3]. Designed bilayers have a good degradation rate, showing 70 % mass loss after one-month real-time degradation without losing the stability of sell-rolled construct [3]. Cell types such as fibroblasts, skeletal muscle cells, and nerve cells have shown high viability and good proliferation. The use of conductive particles in fibrous shape-morphing monolayer showed improved differentiation of nerve cells. Overall fibrous shape-morphing systems have shown promising results for muscle, nerve, and blood vessel regeneration.

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keywords: Electrospinning, self-folding, 4D biofabrication

41883632487

ADDITIVE MANUFACTURING AND ELECTROSPINNING AS A DUAL FABRICATION STRATEGY FOR BIOMIMETIC DRUG-ELUTING BIORESORBABLE STENTS

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Introduction
Bioresorbable stents (BRS) are designed to provide a temporary support to the vessel wall while the structure slowly degrades until completely resorbed. In order to prevent neointimal hyperplasia, antiproliferative drugs such as everolimus are loaded into stents, although endothelialization may be delayed due to long release periods [1]. Additive manufacturing (AM) and electrospinning (ES) are separate approaches to manufacturing scaffolds for a variety of tissue engineering applications. Previously, we have developed a versatile AM fabrication strategy for stents by using polymeric inks and direct-writing onto a rotating cylinder that allows patient-specific customization [2].
The aim of this work is to combine AM and ES to generate new biomimetic drug-eluting BRS for cardiovascular applications.

Methodology
Poly-L-lactic acid (PLLA) and poly(lactic-co-caprolactone) (PLCL) stents were obtained by cylindrical printing onto a rotating mandrel with 3 mm in diameter [2]. The ink consisted in a solution of PLLA (PL 65, Purac) or PLCL copolymer (PLC 9538, Purac) in chloroform at 10% w/v and 12.5% w/v, respectively. Inks were further modified with the addition of antiproliferative drug everolimus at 2 wt.% and 4 wt.% with respect to polymer content. An alternative drug-loading method was developed by means of ES by dissolution of PLCL pellets in chloroform and ethanol (7:3 ratio) at 6.25% w/v with everolimus at 35 wt.% with respect to polymer content. 3D-printed stents were mounted onto a grounded collector mandrel showing both rotation and longitudinal movement. Obtained drug-loaded scaffolds were incubated in 5 mL of release medium (0.7% Triton X-405 in 0.01 M potassium phosphate buffer pH 6 at 37°C in 7% acetonitrile) to evaluate everolimus release by HPLC [3]. Finally, in vitro cell migration studies with HUVECs and SMCs were performed.

Results
Biomimetic drug-eluting BRS were successfully fabricated either by AM or by combination of AM and ES techniques. On the one hand, PLLA and PLCL stents showed drug entrapment within stents' struts. Everolimus release assays showed initial fast release due to surface-available drug followed by a sustained release over 4 weeks. Drug release was subjected to polymer degradation and found to be higher for PLCL stents than for PLLA stents. On the other hand, ES resulted in a homogeneous coating wrapping the entire stent. Although ES-coated BRS showed the same release trend, everolimus release rate was found to be higher than for AM drug-loaded stents. Moreover, fiber orientation and everolimus release of AM/ES BRS directed HUVECs and

SMCs adhesion, proliferation and migration.

Conclusions
Two different PLLA and PLCL drug-eluting BRS were successfully fabricated by means of AM or AM/ES combined techniques. The AM/ES BRS resulted in a fiber-coated drug-loaded biomimetic BRS able to inhibit neointimal hyperplasia while controlling endothelialization.

Acknowledgements
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keywords: Additive manufacturing, drug-eluting bioresorbable stents, poly-l-lactic acid, electrospinning, endothelialization

31412718999

ANTIBACTERIAL RIFAMPICIN-LOADED ELECTROSPUN POLYCAPROLACTONE MEMBRANES FOR URETERAL REGENERATION

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Introduction
With the aim of restoring both the structure and the function of the damaged ureter, the most common medical approaches involve the use of autologous tissue or cells transplants. However, there are many complications that hamper a total or even partial ureter regeneration. Therefore, ureteral regeneration is still a medical unresolved challenge. In this field, electrospun scaffolds are becoming to be considered as a valid alternative to transplants thanks to their biocompatibility, extracellular matrix (ECM) mimicry, slow biodegradability, suitable mechanical properties, and to their ability to sustain cell adhesion and proliferation. To discourage infections of the urinary tract (UTIs) and medical device-related infections (MDRIs), it is important to implement the scaffolds with antibiotic molecules. Rifampicin was found to be effective against the most represented pathogens of UTIs and MDRIs, respectively E. coli and S. aureus. In this work, antibacterial electrospun polycaprolactone-based tubular scaffolds enriched with rifampicin were produced, characterized and proposed as promising candidates for ureteral regeneration.

Methodology
Electrospun polycaprolactone (PCL) and polycaprolactone-rifampicin (PCL/Rif) membranes were obtained and characterized. Given the hydrophobic nature of PCL, all membranes were treated with air-plasma cleaning process. Successively, the wettability was evaluated on both the groups of membranes. The membranes were aged in Simulated Body Fluid (SBF) at 37 °C and then analyzed by uniaxial tensile tests and SEM imaging in order to assess their stability. Rifampicin release kinetic was assessed by means of UV spectrophotometry. To investigate antibacterial efficacy of PCL/Rif membranes, E. coli and some bacteria strains belonging to the “ESKAPE” (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and Enterobacter spp.) group were used. Moreover, the biocompatibility of membranes was tested evaluating urothelium cells (UCs) adhesion and proliferation. Material cytotoxicity was assessed evaluating Lactate Dehydrogenase (LDH) release from UCs.

Results
The PCL membranes produced in this work present homogeneous nanofibers without surface defects. The addition of Rif results in a decrease of the average nanofiber diameter. PCL-membranes treated with plasma and PCL/Rif-loaded resulted to be very hydrophilic unlike untreated PCL membranes. The mechanical characterization, carried out with uniaxial tensile tests, showed a proper stability of the membranes aged in SBF, in terms of deformation at break, elastic modulus and maximum stress and a mechanical behavior suitable for ureter regeneration. The evaluation of the Rif release kinetics showed a burst release of rifampicin in the first 24 hours followed by a release decay. PCL-based membranes were able to sustain UCs adhesion and proliferation over time (confirmed by SEM investigation), but only the ones treated

with Rif were able to inhibit bacterial proliferation. Moreover, the absence of cytotoxic effects of Rif was demonstrated by means of LDH assay.

Conclusions

The satisfactory stability, mechanical, biological and antibacterial properties of the electrospun PCL membranes here produced and characterized set the basis for the development of a tubular scaffold which is promising in providing a valid alternative to transplants practice for the ureteral regeneration. Further in vivo analyses are needed to test and characterize these PCL based tubular scaffolds.

keywords: Antibacterial, Electrospinning, Polycaprolactone, Rifampicin, Ureteral regeneration.

62825459928

CONTROLLING CELL RESPONSES WITH SURFACE POTENTIAL ON ELECTROSPUN POLY(L-LACTIDE) (PLLA) SCAFFOLDS PRODUCED WITH POSITIVE AND NEGATIVE VOLTAGE POLARITY

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Abstract
Cell responses are essential for all tissue regeneration processes. Interaction between cell and material results from biomaterial surface properties such as geometry, wettability, stiffness, or roughness1.
Most studies focus on the surface topography to control cells, however, another crucial parameter is surface potential2. Electrospinning is a commonly used method to produce polymer fibers with tailored surface properties such as voltage polarity. In electrospinning, fibers are produced by drawing charged jets of polymer solution in an electrostatic field between needle and collector with an applied potential difference. On the surface of the polymer solution and the jet charges (positive or negative) accumulate, which contributes to the reorientation of polymer chains pieces or functional groups by electrostatic interactions3. Surface potential is a significant factor of biomaterials regulating cell adhesion and proliferation. In our research, we use poly(L-lactide) (PLLA) to electrospun scaffolds using positive and negative voltage polarity to control their surface potential.
The properties of fibers are verified with scanning electron microscopy (SEM), surface potential with Kelvin probe force microscopy (KPFM), and compared with zeta potential measurements. Finally, the produced scaffolds were used in the osteoblast cell culture (MG-63 cell line) studies and analyzed with confocal microscopy. The result show possibility to control anchoring and proliferation of cells in PLLA. Manufactured with positive and negative voltage polarity to tune their surface potential.

Acknowledgments
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keywords: Electrospinning, poly(L-lactide) (PLLA), cell responses

20941877139

DEGRADATION PERFORMANCE OF A NEW MECHANICALLY REINFORCED DEGRADABLE PHEMA FOR TISSUE ENGINEERING APPLICATIONS: FROM IN VITRO TO IN VIVO

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Development of degradable blood contacting devices (BCDs) is often associated not only with weak mechanical properties and high molecular weight of the degradation products, but also with long-term thrombogenicity issues. Poly(2-hydroxyethyl methacrylate) (pHEMA) emerges as a promising hydrogel to be used in BCDs mainly due to its bio/hemocompatibility and non-fouling character. However, aiming tissue engineering strategies, it is essential to overcome the lack of degradability presented by this hydrogel.
Thus, we aim to develop a degradable pHEMA (d-pHEMA) hydrogel to be used in a post-implantation tissue engineering approach, namely as BCDs. For that, a hydrolytically degradable crosslinking agent, pentaerythritol tetrakis(3-mercaptopropionate) (tetrakis) was copolymerized with pHEMA to allow its degradation, while oxidized graphene-based materials (GBMs) were explored as nanofillers to potentiate its mechanical performance. The hydrogel physicochemical and biological performance was evaluated, including in an in vivo rat subcutaneous implantation model (for the most promising conditions). In vitro results showed that inert and biocompatible pHEMA hydrogel was turned into a degradable material by incorporation of 0.25% (v/v) tetrakis. For higher tetrakis concentrations, low mechanical properties were achieved, ultimately not leading to film formation (>1% (v/v)). In situ addition of different GBM types allowed an improvement in the mechanical properties – 1wt% few-layer graphene oxide with 5 μm lateral size (M5ox) increased the ultimate tensile strength up to 0.2 MPa (4x higher than d-pHEMA) – and tuning of the in vitro degradation time in PBS, ranging from 2-4 months.
Notably, the intrinsic properties of pHEMA were kept, namely water uptake (» 60% of dry weight), wettability (» 40° contact angle) and short and long term cytocompatibility (24h and 6M extracts). The anti-adhesive properties were confirmed, with no adhesion of human umbilical vein endothelial cells to d-pHEMA nor d-pHEMA/GBMs hydrogels' surface, after 1 and 7 days. Similarly, there is no platelet adhesion to the surface of the films. Such features are promising when envisioning the production BCDs, suggesting thrombus formation could be minimized. Upon subcutaneous implantation of pHEMA, d-pHEMA and d-pHEMA/M5ox in inbred Sprague

Dawley rats for 6M, no signs of inflammation or infection were observed at the implantation areas, with macroscopic photos confirming stability of pHEMA, and revealing a high degradation of the d-pHEMA (starting at 3M and almost complete after 6M). Contrarily, d-pHEMA/M5ox did not show significant degradation, in contrast with the in vitrodegradation results, which may be explained by a lower hydration and therefore, lower hydrolytic action when implanted.
The herein described newly developed degradable hydrogels have considerable potential as scaffolds for tissue engineering applications, with the greater amount of tetrakis leading to higher degradation. Depending on the envisioned application, increase of mechanical properties and delay of the degradation time can be modulated by the incorporation of few-layer graphene oxide. For example, d-pHEMA/M5ox are degradable, but keep their stability for at least 3 months, which suggests an appropriate timeframe for tissue regeneration in the vascular context.

keywords: Hydrogel, graphene based-materials, subcutaneous implantation, biocompatibility, blood- contacting devices

62825426168

DEVELOPMENT OF BIOMIMETIC TYMPANIC MEMBRANE SUBSTITUTES FOR THE TREATMENT OF CHRONIC PERFORATIONS

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Introduction
Tympanic membrane perforations (TMP) represent a common cause of visit to the ENT clinic, usually due to recurrent infections or trauma. The rate of recovery for each perforation depends mainly on the size of the perforation and the secondary infections developed. Although most TMPs heal spontaneously, some patients can develop a chronic defect. The traditional technique used in the treatment of TMP, known as myringoplasty, uses temporalis muscle fascia or tragal cartilage perichondrium, with its associated with patients' morbidity and high healthcare expenses. Thus, there is growing interest in the application of biocompatible materials such as scaffolds for regeneration. The aim of this study is the development and validation of a protein-based biomaterial scaffold to be used as a carrier in tympanic membrane restoration.

Methodology
Scaffolds were designed based on porcine gelatin. Subsequently, the scaffold was characterised by water uptake, water vapour transmission rate and degradation degree analyses. Moreover, mechanical properties were evaluated by means of puncture and mucoadhesion tests. Finally, the biocompatibility of the scaffold was analysed by exposition to a fibroblast cell line. In light of these results, a pilot in vivo study was conducted in a rat model with chronic TMP in order to study the tympanic membrane (TM) substitute integration, regeneration and functionality. For that aim, first, the perforation was created on both TMs by applying mitomycin C before the incision and dexamethasone after the procedure. The perforations were evaluated during 8 weeks by otoendoscopic observation and those who became chronic were included. The designed scaffold was placed into the study ear after margin scar tissue removal covering the previously created defect. Afterwards, the perforation was being monitored for 2 months by closure level measurement and blood analysis. After this time, the animal will be sacrificed, and tissue will be removed from the implantation site to evaluate the regeneration degree and the response of the graft in the host.

Results
Regarding the in vitro characterisation, the substitute showed adequate hydration and permeability properties, and did not degrade when exposed to water following 6 months. Mechanical properties, pressure resistance and adhesion capacity, of biomimetic substitutes were similar or superior to those reported in the literature for native TM. The scaffolds were fully biocompatible with no relevant affection of cell viability over one-week period.
Concerning the in vivo study, the chronic TM defect was successfully created in the 60% of the cases. After scaffold placement, otoscopic observations seem to show integration of the TM substitute without apparent swelling.

Conclusion
The designed gelatin-based biomimetic TM substitute showed intrinsic and functional properties in vitro that make it suitable as a support for the regeneration of chronic TMP. Due to its intrinsic

properties, it allows the maintenance of a suitable environment for wound closure and its functional properties facilitate manipulation and adaptation to the type of pathology. In view of the results compiled from the in vivo trial, this substitute could allow TMP closure without causing any adverse response.

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keywords: tympanic membrane, tympanic perforation, scaffold, gelatin.

62825408328

EXPLORING THE STRUCTURAL, MORPHOLOGICAL, AND CHEMICAL PROPERTIES OF SPIDER SILK CRUCIAL FOR ITS SUCCESS IN NERVE REGENERATION

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Introduction: Spider silk (SPSI) as one of nature's most fascinating materials has attracted vivid attention due to its remarkable performance in tissue regenerative applications by supporting nerve growth and nerve regeneration. Particularly for supporting the regeneration of large nerve defects, the biomaterial has proven to be incomparably successful. The material's variability and the difficulty to harvest it in large quantities constitute major limitations in translating the fiber into clinical practice. For this reason, the search for possible SPSI analogues for applicability in human medicine is of tremendous scientific and clinical interest. To pave the way toward this, an investigation of structural, morphological and chemical properties of different SPSIs together with an assessment of their nerve regenerative potentials is necessary.

Methodology: This systematic study correlates varying in vitro performances of different SPSI to the silk's material properties. Therefore, primary rat Schwann cells (rSCs) were cultured on SPSI of various species and live cell imaging was performed to assess the migratory potential of cells by tracking them using ImageJ. The extent of culture's purity and proliferation was examined by multicolor immunofluorescence stainings. Liquid chromatography - mass spectrometry and atomic force microscopy were employed to elucidate SPSI's primary protein structure and its morphology, respectively.

Results: The results showed that rSCs can adhere and migrate along SPSI, with deviating velocity depending on SPSI type. Multicolor images of rSCs stained for Sox10 and S100 in combination with DAPI indicated a rSC culture purity over 95%. Furthermore, the proliferation of rSCs on SPSI was evaluated with an EdU staining. These differences in the cell behavior on SPSI were correlated to the silk's morphology and primary protein structure.

Conclusion: So far, very little is known about the interactions between SCs and SPSI, rendering the well-aimed and targeted improvements of the natural silk and replacement with customed artificial fibers, tailored to specific applications, challenging. Our results demonstrated variations in the regenerative potentials of SPSI and showed that it is possible to use the natural differences between the native silks of diverse spider species to better understand the interactions between silk and cells.

keywords: spider silk, peripheral nerve regeneration, atomic force microscopy, liquid chromatography - mass spectrometry

31412704959

FABRICATION OF POLY(HEMA-CO-MMA) POROUS SCAFFOLD WITH HIGHLY BIOCOMPATIBILITY FOR SOFT TISSUE REGENERATION

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*Purpose/Objectives: Scaffolds with a tissue-like characteristics and suitable physical properties are critical for tissue engineering and regeneration. 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were commonly used for biomaterials because of their high biocompatibility and stability in the host tissue. Herein, we report porous poly(HEMA-co-MMA) scaffold for tissue engineering and soft tissue regeneration.

*Methodology: Porous poly(HEMA-co-MMA) scaffolds were developed by combining phase separation and cross-linking for soft tissue regeneration. The phase separation technic was used in the HEMA-MMA mixed solution to generate irregular micro-sized porous via polymerization. During the polymerization process, the HEMA and MMA are polymerized to poly(HEAM-co-MMA) and separated from other solvents to form interconnected porosity. The mechanical characteristics, biocompatibility and inflammatory response of the fabricated porous scaffolds were evaluated by SEM observation, cytotoxicity assay, inflammatory-related cytokine assay and rat subcutaneous implantation experiment.

*Results: The FACS assay showed that the porous scaffolds did not induce the expression of inflammatory markers like as iNOS, IL-6, TNF-α in the macrophages significantly. The WST assay and SEM image analysis for the co-cultured with porous scaffold and human dermal fibroblast showed good cytocompatibility and successful cell adhesion and cell proliferation. Also, histological analysis of rat subcutaneous implant evaluated the biocompatibility of the porous poly(HEMA-co-MMA) scaffolds.

*Conclusion/Significance: These results demonstrated that porous poly(HEMA-co-MMA) scaffolds could be fabricated and provide suitable mechanical properties and biocompatibility as biomaterials for tissue engineering. By extension, the present results suggest a promising approach of synthetic biomaterials in soft tissue engineering.

keywords: Scaffold, poly HEMA-co-MMA, biocompatibility, soft tissue

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FABRICATION OF POLY(HEMA-CO-MMA) SCAFFOLD HAVING SURFACE ROUGHNESS AND MODULUS FOR SOFT-TISSUE ENGINEERING

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*Purpose/Objectives: The surface roughness, morphology and modulus of the scaffold are critical factors in tissue engineering and biomedical applications. In this study, we developed a scaffold suitable for the soft tissue with high modulus and surface modifications for excellent cell-adhesion and cell spreading by the poly(HEMA-co-MMA) concentration-responsive characteristics.

*Methodology: Porous scaffold for suitable roughness and mechanical property was fabricated by the polymerization of various ratio of HEMA and MMA. The mechanical characteristics include the surface roughness, modulus, pore size and cell toxicity, adhesion and proliferation activity depend on the ratio of HEMA and MMA were evaluated.

*Results: The surface morphology and the pore size of the scaffold were controlled by changing the ratio of HEMA and MMA. in particular, poly(HEMA-co-MMA) scaffold with 1-5 KPa which applicable to soft-tissue engineering were developed by the HEMA 90mol%, MMA 10mol% of total polymer content. The results of in vitro cell response assay showed the difference in cell adhesion and spreading on the scaffolds according to surface roughness and mechanical properties.

*Conclusion/Significance: In this study, the porous scaffolds made of poly(HEMA-co-MMA) exhibit surface roughness and mechanical properties suitable for soft tissue regeneration and tissue engineering.

keywords: scaffold, hydrogel, soft-tissue engineering

52354540419

HEALING-TRIGGERING BIOMATERIALS FOR FETAL MEMBRANE REPAIR

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Introduction: Minimally-invasive prenatal interventions which aim to ameliorate fetal developmental defects have become a clinical reality and are currently performed for a variety of life-threatening complications. However, in up to 30% of the cases, the intervention translates into preterm birth and its associated negative consequences. The puncture created in the fetal membranes (FMs) during fetoscopy and their reported inability to heal might play a role in the risk for iPPROM. Despite promising, none of the investigated approaches has been clinically translated, and there is currently no clinical strategy to prevent iPPROM after a fetoscopic intervention. We have earlier shown that regeneration-inducing factors, such as platelet derived growth factor (PDGF)-BB can promote the migration and proliferation of FM cells when encapsulated in a biomaterial. Here, to elucidate the potential such biomaterial for FM repair in a clinically relevant setting, we investigated the healing-triggering capacity of such biomaterial in an ovine model.

Methods: To investigate the effect of PDGF-BB to elicit a healing response of FMs, we established a FM defect model in pregnant sheep. In this novel in vivo model, we applied an umbrella-shaped nitinol implant loaded with our previously engineered poly(ethylene glycol) (TG-PEG) biomaterial that released PDGF-BB. At explantation, we performed macroscopic examinations as well as immunohistochemistry analysis of the implants.

Results: The comparison of empty or growth factor-loaded implants shows that platelet-derived growth factor (PDGF-BB) promoted a healing response consisting of angiogenesis, immune cells, and migration, proliferation and ECM deposition in the implanted biomaterial.

Conclusion: This study is a first proof-of-concept that TG-PEG hydrogels loaded with PDGF-BB, by triggering cell recruitment and proliferation from the myometrium and the vicinity of the FMs, might be able to heal FM defects. Longitudinal studies lasting until the time of delivery will be required to understand the long term healing response of the FMs.

keywords: fetal membranes, iPPROM, fetal membrane healing, sheep model, fetoscopy

52354563048

MCSS SUPPORT A LAMELLA-LIKE TWISTING ORIENTATION OF COLLAGEN WHEN CULTURED ON ALIGNED ELECTROSPUN POLYCAPROLACTONE FIBRES

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Introduction
It has been shown for many connective tissue types that substrate orientation can control collagen and ECM deposition. This has been used to engineer tissues with aligned fibril structures such as tendon. In cortical bone, collagen is organised into a lamellar structure with highly aligned collagen sheets packed into a “twisted plywood” configuration, however, reproducing this in vitro proves challenging. In a previous unpublished study within our laboratory, mature late-osteoblast-like cells, MLO-A5, cultured on aligned polyurethane fibres deposited collagen along the direction of the fibres. Above the scaffold, collagen was deposited in a direction offset to the initial layer, continuing in subsequent layers, creating a lamellae-like twisted-plywood structure. In this study we aimed to test whether human cells at an early stage of osteogenic differentiation would deposit collagen exhibiting a twisted plywood deposition behaviour, therefore providing a model system of laboratory-grown lamellae for use as a model of bone formation.
Methodology
Aligned and non-aligned polycaprolactone (PCL) fibers were fabricated by electrospinning and treated with air plasma. hTERT Y201 immortalised human MSCs [1] were seeded directly onto the scaffolds and supplemented with osteogenic induction media. On days 14, 21 and 28 collagen was imaged using second harmonic generation (SHG). Collagen alignment was analysed using the ImageJ “Directionality” Plug-in. Samples were also stained for calcium and collagen deposition using Alizarin Red stain (ARS) and Sirius Red stain (SRS).

Results
Collagen was initially shown to orientate along the direction of the aligned PCL fibres. By D21 orientation patterns that varied with depth could be observed, with collagen layers having a twist in the anti-clockwise direction, compared with their substrate layer with an average change in orientation of 16.53° ± 13.22. By D28, the orientation had shifted from the initial direction of the PCL fibres by 53.1° ± 15.6. For non-aligned scaffolds there was no noticeable collagen directionality, or difference between layers. Mineral and collagen staining showed that there were no significant differences in overall quantity of matrix produced within either scaffold type.

Conclusions
Attempts to tissue engineer bone generally result in a disorganised matrix, comparable to that of woven bone or a healing fracture. In this work, it was shown a possibility to guide the collagen into a structure comparable to the cortical bone lamallae. Interestingly examinations of lamellae organisation have shown periodicities of about 5-7μm, and gradual change of fibril direction, varying from 10-60° from the orientation of the lamellae at the osteon’s centre. Within this work, the change in collagen direction also remained within this range, but occurred over larger depths of 35-45μm[1] . Further work should explore the mechanisms, as this may provide an insight into

how collagen organises into tissue-specific structures.

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Acknowledgements
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keywords: cortical bone, in vitro models, MSCs, electrospinning, collagen

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NANOCOMPOSITE POLYMERIC THIN FILMS FOR BOOSTING SKELETAL MUSCLE CELL DIFFERENTIATION

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Introduction
Skeletal muscle (SM) tissue engineering aims to achieve a mature SM tissue in vitro, by combining precursor cells, suitable substrates and the right stimuli to facilitate cell growth and differentiation into the desired phenotype. In this field, polymeric thin films are attractive due to their small thickness (from hundreds of nm to some µm), flexibility, adhesiveness, permeability and similarity to natural biomembranes. [1]
Moreover, their tunable stiffness and the possibility to create micropatterns on their surface by nano and micro-fabrication techniques make them promising substrates to reproduce the mechanical and geometrical environment typical of natural SM tissue. In this work, we developed novel nanocomposite microgrooved polymeric thin films able to boost the skeletal muscle differentiation of C2C12 cells.

Methodology
Solutions of poly(styrene-block-butadiene-block-styrene) (SBS) dissolved in tetrahydrofuran and polycaprolactone (PCL) dissolved in dichloromethane were prepared at 40 mg/mL. Barium titanate nanoparticles (BTNPs) (diameter: 300 nm) were added at different concentrations (0.01% w/v, 0.1% w/v, 1% w/v) and the final solutions were spin-coated (2000 rpm, 20 s) over a microgrooved (10 µm parallel channels) PDMS mold. A solution of PVA was cast over the thin films as supporting layer to detach the films and then dissolved in deionized water, thus obtaining freestanding microgrooved thin films.
The films were characterized in terms of topographical (stylus based profilometer and optical profilometer) and mechanical (nanoindenting through AFM) properties and used as substrates for adhesion and differentiation of murine myoblasts (C2C12, seeding density: 50000 cells/cm2). During the differentiation period (7 days), the differentiation medium (DMEM + 1% FBS + 1% P/S +1% ITS) was renewed every day. At the end-point, the samples were stained for F-actin and nuclei and analyzed through a confocal microscope, to quantify myotubes dimensions and fusion index.

Results and conclusions
The thin films obtained showed an average thickness of 700 nm (SBS) and 1.3 µm (PCL). Their channels were 10 µm-wide and 1 µm-high. Their elastic modulus was 60 MPa (SBS) and 26 MPa (PCL), respectively.
Immunostaining evidenced the development of aligned myotubes that uniformly covered the sample area. PCL films showed a fusion index from 38.065±5.982% (BTNPs 0%) to 31.824±7.592% (BTNPs 1%) and SBS films from 62.018±3.382% (BTNPs 0%) to 65.146±7.942% (BTNPs 1%). These high values confirmed a very good level of differentiation, without significant differences between doped and not-doped films of the same polymer.
As a perspective, the piezoelectric properties of barium titanate nanoparticles will be exploited in combination with ultrasound stimulation to further boost cell differentiation, especially in SBS samples.

Acknowledgment
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keywords: Skeletal muscle tissue engineering, polymeric thin films, piezoelectric nanoparticles

62825455377

POLY(L-LACTIDE-CO-GLYCOLIDE) MEMBRANES SURFACE-MODIFIED WITH RGD-GRAFTED POLY(2-OXAZOLINE) FOR GUIDED TISSUE REGENERATION IN PERIODONTOLOGY

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Introduction
Guided tissue regeneration (GTR) is a surgical procedure that specifically aims to regenerate soft and hard periodontal tissues when they are irreversibly destructed. GTR requires special porous barrier membranes, preferentially degradable, capable of preventing soft tissue infiltration into the bone defect and simultaneously supporting bone regeneration. The aim of this study was to produce a degradable poly(L-lactide-co-glycolide) (PLGA) membrane with appropriate microstructure, i.e. less porous on the surface intended to be in contact with the gum, while being more porous on the surface contacting the bone tissue defect to promote osteogenic cell adhesion, proliferation, and differentiation, and thus bone tissue restoration. In addition to microstructural cues, we provide our membranes with arginine-glycine-aspartic acid (RGD) motifs, which may act as biochemical cues supporting specific integrin-mediated cell adhesion.

Methodology
The membranes were produced using poly(L-lactide-co-glycolide) (PLGA, 85:15, Mn = 100 kDa, d = 1.9). Poly(2-metyl-2-oxazoline-b-2-butyl-2-oxazoline-b-2-methyl-2-oxazoline) (POx), was modified with an RGD derivative with 6-aminohexanoic acid (POx-RGD). To obtain the membranes, we co-dissolved PLGA, PEG, and POx_RGD in DCM, solvent-casted, dried, followed by PEG leaching [1]. Raman spectroscopy, FTIR-ATR, and XPS were used to characterise all ingredients and the membranes. The membranes were also characterized using SEM; mechanical properties, susceptibility to degradation, wettability, and surface free energy were also assessed. Osteoblast-like MG-63 cells were cultured for 4, 24 and 96 h on the membranes and analyzed by metabolic activity and live/dead tests, as well as by phalloidin/DAPI fluorescent staining to visualize cell morphology and cytoskeleton reorganization.

Results
PLGA, PEG, POx, RGD, and POx_RGD were characterized using Raman, FTIR-ATR, and XPS spectroscopic techniques. Detailed analysis of the spectra confirmed that RGD was successfully coupled with POx. The membranes for GTR were obtained by phase separation and preferential adsorption of POx_RGD molecules at the PLGA/PEG interface with POx_RGD exposed to hydrophilic PEG, followed by solvent evaporation and PEG leaching. The membranes had an asymmetric microstructure, as shown in the SEM pictures of both the surfaces and cross-sections; the glass-cured surface was more porous and was characterized by a higher surface area as compared to the air-cured surface. XPS and FTIR-ATR studies confirmed that POx_RGD was immobilized on the membrane surface; however, this modification practically did not

influence the surface wettability and surface free energy values. In vitro tests showed that the POx_RGD-modified PLGA membranes supported osteoblast-like cell adhesion, proliferation, and viability to the highest extent, compared to membranes without modification or modified only with POx.

Conclusion
The one-step phase separation process between PLGA, PEG, and POx_RGD dissolved in DCM, followed by drying and leaching of PEG, resulted in asymmetric PLGA membranes with enhanced biological properties, which could be considered for the guided tissue regeneration technique in periodontology and bone tissue engineering.

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keywords: poly(L-lactide-co-glycolide), poly(2-oxazoline), RGD sequences, phase separation, guided tissue regeneration (GTR)

73296333244

POSTPRODUCTION PROCESSING OF ELECTROSPUN POLYCAPROLACTONE FOR OESOPHAGEAL TISSUE ENGINEERING

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Oesophageal cancer is the sixth most fatal cancer in worldwide and often requires surgical removal alongside chemo- or radiotherapy. The surgical treatment options available are highly invasive and have a large impact on a patient's quality of life. Oesophageal tissue is complex and has poor regenerative capacity meaning further surgical repair is usually required after oesophagectomy, often using stomach or colon to replace lost oesophageal tissue (1). Common surgical repair techniques have high rates of mortality and are associated with many complications (2).
We are developing a biomaterial scaffold to be used as part of a regenerative medicine approach to the repair of oesophageal tissue. This has the potential to improve many patient outcomes by removing the need for a further surgical procedure to obtain donor tissue, which is often not able to restore full function to the area.
Electrospinning is often used to produce biomaterial scaffolds for regenerative medicine as the resulting nanofibres are similar in structure to the extracellular matrix. Aligned fibres in particular have been shown to increase migration into a wound site from surrounding tissue (3). While there are many benefits to using electrospun synthetic polymers as a biomaterial scaffolds, they usually must undergo significant post-production processing to maximise their suitability.
We have used electrospinning to produce sheets of aligned polycaprolactone (PCL) nanofibres with a fibre diameter as low as 100 nm. This fibre diameter is similar to that being used for successful tissue engineering by several groups, and within the range of the oesophageal ECM fibre diameter (28-165nm). PCL was chosen for this study because it has shown promise as an implantable biomaterial for several regenerative medicine applications, including in the form of electrospun nanofibres. PCL nanofibres are biocompatible and biodegradable, but not very bioactive or hydrophilic.
We are investigating the effect of several postproduction processes on the activity of oesophageal fibroblasts when seeded onto the electrospun PCL scaffold. We have functionalised PCL nanofibres with fibronectin via hydrolysis, which also increases the wettability of the scaffold (4). We have developed a method to imprint micro scale (25 um) grooves onto the surface of a sheet of PCL nanofibres allowing us to investigate how cells respond to different scales of topographical alignment.
It is also important when developing a biomaterial scaffold to be aware of the mechanical forces that it will experience once implanted into the relevant in vivo environment. We have investigated the effect of four different sterilisation and disinfection methods on mechanical properties of PCL nanofibres, to determine whether they can be used to sterilise the scaffolds without degrading it to the point of no longer being suitable for in vivo implantation.

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keywords: Electrospinning, Oesophagus, Polycaprolactone, Fibronectin, Nanofibres

52354571208

PRETERM HUMAN AMNION COMPOSITION TO INSTRUCT BIOMATERIALS-BASED STRATEGIES FOR THE PREVENTION OF PRETERM BIRTH

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INTRODUCTION
Preterm birth one of the biggest complications in Obstetrics and is the case of life-long morbidities or even mortality of newborns. With advances in fetal therapy, the occurrence of operation-induced failures of fetal membranes (FM) ending in preterm birth is increasing. Responsible for this adverse outcome are the created defects in the FM that do not spontaneously heal. Treatments aiming at the preventive closure these defects have not been successful so far. Understanding the biology of the FM biology at the time of intervention would be essential to develop regeneration-promoting strategies.

METHODS
Term and pre-term amnion biopsies were collected after caesarian section at 36 to 37 gestational weeks (GW) and open fetal surgeries for the treatment of fetuses with spina bifida (GW 21 to 23). Protein extraction was optimized by undertaking a systematic shotgun proteomics approach using a high pH reversed-phase liquid chromatography (RPLC) sample fractionation followed by low pH liquid chromatography-mass spectrometry analysis (LC-MS/MS). Isolated FM cells and tissues were encapsulated in synthetic poly(ethylene glycol) PEG-based biomaterials, treated with selected stimulants and the proliferation and migration of cells was followed.

RESULTS
Over 5000 proteins were identified in the term human amniotic membrane. Differential expression of extracellular matrix (ECM) components were identified in preterm and term amnion. Selected growth factors and ECM components were shown to modify the growth and migration of amnion cells.

DISCUSSION
The high number of identifications demonstrates for the first time the high complexity of this fetal tissue. We identified several core ECM and ECM-associated proteins that can modify the regeneration of the amnion. Integrating the identified signals into new hybrid biomimetic materials could be promising for the treatment of FM defects.

CONCLUSION
To our knowledge, this is the first time that the human amnion protein composition of preterm amnion has been evaluated in a global proteomics approach. This knowledge will instruct the development of therapies for the prevention of preterm birth.

keywords: amnion, preterm birth, biomaterial, proteomic

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SUPERIOR MECHANICAL PROPERTIES OF CELL-LADEN MICROFIBER VIA INCORPORATION OF SILK IN HYALURONIC ACID BASED HYDROGEL

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Cell-based tissue engineering is promising to create living functional tissue. The implanted cellular construct should be evenly distributed cells in the scaffold, maintain high cellular viability as well as mechanical properties in order to actively participate in the regenerative process. Cell-laden filament-like hydrogels have advantageous for tissue engineering and regeneration medicine. However, most of the designed filament vehicles hold weak mechanical properties which hinder their application in specific tissue engineering. We have developed double network silk and hyaluronic acid hydrogel microfiber that generated through microfluidic system with superior mechanical properties and biocompatibility. Cellular microfibers were continuously generated through coaxial double orifice microfluidic device and horseradish peroxidase mediated reaction which conjugated introduce phenolic moieties in the backbone of HA and silk derivatives (HA-Ph&Silk-Ph respectively). The hybrid Silk-Ph/HA-Ph fibers fabricated in micron size through control of outer flow velocity. The tensile strength and maximum stain prepared Silk-Ph/HA-Ph sample was more than four times higher than the single HA-Ph sample which demonstrated significant effect of synthesized silk derivative in hydrogel fiber composition. The proteolytic degradation synthesized fibers manipulated by hyaluronidase and collagenase treatment. Encapsulation process and enzymatic crosslinking did not insert any harmful effect on cell viability and cells maintained their growth ability after encapsulation. We fabricated cellular filament-like tissue from encapsulated cells in Silk-Ph/HA-Ph hydrogel fibers. Together, we believe that the developed composite and method holds great potential in engineering musculoskeletal tissue constructs for applications in regenerative medicine and tissue modeling.

keywords: Silk & Hyaluronic acid conjugated tyramine, Hydrogel microfiber, Mechanical property, Cell encapsulation

94238155089

TOWARDS ADIPOSE TISSUE ENGINEERING USING PHOTO-CROSSLINKABLE GELATIN-BASED BIO-INKS

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There exists a clear clinical need for adipose tissue reconstruction strategies to repair adipose tissue defects which outperform the currently available approaches. The development of biomimetic materials able to promote cell proliferation and adipogenic differentiation has gained increasing attention in the context of adipose reconstructive purposes. Thiol-norbornene crosslinkable gelatin-based materials were developed and benchmarked to the current commonly applied methacryloyl-modified gelatin (GelMA) with different degrees of substitutions focussing on bottom-up tissue engineering [1].
The developed hydrogels resulted in similar physico-chemical properties (gel fractions >90% and mass swelling ratio ~13). The mechanical properties of the hydrogels could be tuned by incorporating more or less crosslinkable functionalities or using different crosslinking techniques (i.e. step-growth ~15kPa vs chain-growth ~30kPa). The biocompatibility (viability >85%) as well as differentiation potential of encapsulated adipose tissue-derived stem cells were analysed through a live/dead assay, Bodipy/DAPI staining, triglyceride assay as well as a secretome analysis. Additional in vivo experiments are currently ongoing assessing the differentiation potential and neovascularisation via ex vivo histology of constructs implanted sub-mammary in mice. Initial in vivo data already showed good vascularisation throughout the construct one month post-surgery via contrast-enhanced μCT imaging.
It can be concluded that the mechanical properties of a biomaterial are of utmost importance with respect to differentiation into the adipogenic lineage. The mechanical cues of GelNB55/SH75 were superior over the other investigated hydrogels. Photo-crosslinkable thiol-ene systems thus offer a promising strategy toward adipose tissue engineering through cell encapsulation compared to the widely used GelMA.

Akwnowledgements
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keywords: Hydrogels; Adipose tissue engineering

41883604488

TUNABLE DEGRADATION AND SELF-POWERED STIMULATION OF PIEZOELECTRIC SCAFFOLD TO MODULATE CHONDROCYTES DIFFERENTIATION FOR CARTILAGE REPAIR

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Generally, articular cartilage disorder at the junction mainly results from constantly repeated dynamic tension/compression effects with aging time. Piezoelectric materials can deliver variable electric signals analogous to native tissues such as bone and cartilage associated with extracellular matrix (ECM) materials. Thus, there is a high need of degradable piezoelectric scaffold, which mimics the dynamic mechanical loading and optimizes the chondrocyte differentiation during the degradation. A degradable aligned electrospun poly-L-lactic acid (PLLA) modified with rGO and PDA fibrous scaffolds with different orientations and surface morphologies (wrinkled and porous) was developed as a biocompatible and degradable piezoelectric scaffold with the self-powered tunable piezoelectricity to modulate cell behavior and cell differentiation of ATDC5 cells by tuning the degradation effect. The electrical output and mechanical properties of the composite fibrous scaffold can be improved by adding rGO and applying mechanical force along the 90° orientation. With changing the degradation behavior, dynamic mechanical loading on the porous PLLA/rGO/PDA fibrous scaffold exhibits significant increase in cell proliferation and secretion of extracellular matrix (ECM). More surprisingly, long-term degradation favored to promote cell differentiation of ATDC5 towards a chondrogenic phenotype due to dynamic mechanical loading, low-intensity electrical stimulation and interconnected porous structural morphology. In contrast, on the wrinkled PLLA/rGO/PDA fiber inducing mineralization with the differentiation of ATDC5 into osteocytes. The modulation of the degraded environment and electrical stimulation of the piezoelectric scaffold offers an effective alternative to influence cell functions, significantly improving the ECM secretion and cell differentiation.

keywords: Electrospinning, Piezoelectric scaffold, Chondrocytes, Self-powered stimulation, Degradation

PS63
Towards automated technologies for organoid-based tissue biomanufacturing

83767267277

AUTOMATED PLATFORM FOR HIGH THROUGHPUT, DEEP LEARNING-BASED SORTING OF SPHERICAL 3D CELL MODELS FOR LIVER TISSUE ENGINEERING.

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Liver disease is a major healthcare challenge, accounting for around 2 million deaths yearly worldwide. Although liver transplantation is the best way to re-establish normal liver function, less than 10% of transplantation needs are currently met. The EU Horizon 2020 OrganTrans consortium aims to address this issue by developing a liver tissue printing platform for transplantation. In this approach, spherical 3D liver models are used as building blocks for the bioprinted liver construct. One critical need is, therefore, a reliable source for large quantities of homogenous and healthy liver spheroids. Major limitations of current existing solutions are throughput efficiency and classification of complex objects where standard criteria, such as size and circularity, do not represent a sufficient measure of quality.
To answer this need we developed an automated deep learning-based sorting platform which allows for the screening of spheroids after formation. The platform includes imaging, classification, and individual sorting of spheroid. The imaging module consists of a brightfield microscope and is compatible with numerous microwell plates to image the spheroids directly in the culturing plate. The deep learning network is trained by experts and allows for the classification of healthy and unhealthy spheroids. A capillary needle enables removal of individual unhealthy spheroids, and the remaining healthy spheroids are harvested and concentrated in a Falcon tube. The platform is compatible with biosafety cabinets to ensure sterility. The system proved to successfully sorts 20 000 spheroids within 1.5 hours and with a classification accuracy of > 98% for both HepG2 monoculture spheroids and tri-culture liver spheroids.
The developed platform performs reliable sorting of complex 3D models, such as monoculture and co-culture spheroids, for which standard sorting criteria lack relevancy. This system represents a flexible solution for applications in regenerative medicine where large quantities of standardized spheroids are needed.

keywords: tissue engineering, automated sorting, deep learning

52354516564

AUTOMATED QUANTIFICATION OF ORAL MUCOSA STROMA COMPONENTS THOROUGH MACHINE LEARNING ON HISTOLOGICAL SAMPLES. A POTENTIAL TOOL IN TISSUE ENGINEERING

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Introduction: Over the last decade, an increasing relevance has been given to the use of semi-quantitative and quantitative methods to evaluate histological and immunohistochemical images through image analysis software. Most of this software only require image input. However, the usability of classic image processing, including classic segmentation algorithms, is limited. Machine learning could provide a new tissue engineering tool that may allow a feasible, objective, and automated approach that serve as quality control of bioengineered tissues. In this context, the aim of this study is to develop a supervised machine learning approach for the accurate and automatic quantification of collagen fibers in human oral mucosa substitutes generated by tissue engineering and stained with Picrosirius red for collagen detection.

Methods: Human oral mucosa substitutes were generated by tissue engineering. First, oral mucosa keratinocytes and fibroblasts cell cultures were established from small oral mucosa biopsies. Then, a stromal substitute was fabricated using 0.1% fibrin-agarose and epithelial cells were cultured on top1. Native oral mucosa samples were used as control, and both types of samples were stained with Picrosirius red. Then, a neural network was developed and trained from scratch using 10 large histological images. During training, we took random patches and applied different types of data augmentation to them. Following this process, we were able to create in real time an uncountable amount of unique small patches from the original images. Once trained, we tested the network in a test set of histological images. To obtain the mask of a specific area, sub-masks were obtained and finally combined to obtain the entire area.

Results: Application of the automated quantification system developed here allowed us to accurately identify the target structures in each histological image. Under leave-one-out cross-validation over the histological images, the method yielded over 90% pixel accuracy and collagen precision in training and over 85% in pixel accuracy and collagen precision in set.

Conclusions: The proposed machine learning model based in neural networks was able to segment collagen in histological images based on semantic information, instead of the more classic color segmentation used in the field. The developed models demonstrate to be more robust to color outliers and is able to produce a better segmentation. Furthermore, the segmentation produced by the network is fully automatic, which also reduces the tedious process of having to manually fine-tune a color range to get a correct segmentation. This quantitative and automated approach open a new window on quality control techniques for bioengineered human oral mucosa tissues.

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keywords: Machine Learning, Histology, Quantitative Analysis, Collagen

83767243197

MAPPING THE PROTEIN SECRETOME OF BONE FORMING CARTILAGE MICROTISSUES ACROSS DONORS

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Introduction: Bottom-up developmental engineering is showing promising results in skeletal tissue engineering1. In this approach, microtissues are formed from periosteal cells, the main contributors in the formation of the cartilaginous fracture callus. These microtissues can be chondrogenically differentiated towards hypertrophy resulting in a cartilaginous template in vitro which can be remodeled after implantation resulting in bone ossicles mimicking closely fracture healing. However, further clinical translation of these promising implants is hampered by (1) a lack of information on donor variation, and (2) a lack of non-invasive potency monitoring techniques. In this study, the protein secretome of bone-forming microtissues was monitored over time for 6 individual donors to extract secreted end-point efficacy biomarkers.

Methods: Human periosteum-derived cells from 6 donors were seeded in non-adherent microwells (Aggrewell TM 800, STEMCELL Technologies) to form microtissues of 1000 cells each. These microtissues were cultured for 21 days in a low-protein chemically defined chondrogenic medium. After 5 hours, 7, 14, and 21 days microtissues were collected for histological staining and DNA quantification, and their culture supernatant was analyzed by mass spectrometry for identification of the protein secretome. Then, 600 microtissues were fused and implanted ectopically for 4 weeks in nude mice to assess bone-forming potency.

Results: Histological analysis of microtissues showed a clear distinction between 3 donors with a high production versus 3 donors with low production of extracellular matrix, generating large and smaller microtissues. These differences were related to the gender of the donors. Similar differences were seen in the total amount of soluble protein in their culture supernatant (38.8±6.1 µg/mL v.s. 22.1±1.4 µg/mL, p = 2.5 x 10-5). A total of 172 proteins were detected in the culture supernatant by LC-MS/MS. Interestingly, most proteins were already detected after 7 days and showed a decrease towards day 21. However, when implanted, cartilaginous implants resulted in the formation of bone ossicles for all donors, showing high protocol robustness. Therefore, we identified 10 secreted proteins that were commonly found for all donors that could be promising candidates for bone-forming potency biomarkers.

Discussion: We carried out a series of orthogonal quality characterization studies for human donor-derived cartilaginous microtissues evaluating also bone-forming capacity in vivo. Furthermore, we used high-sensitivity proteomics for mapping the secretome during chondrogenic differentiation. This is promising for the identification of biomarkers that will enable the implementation of quality-by-design strategies by predictively controlling the microtissue differentiation processes in vitro to ensure skeletal defect regeneration upon implantation.

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keywords: Secretome, Donor variability, Biomarkers

PS64
Understanding and preventing early inflammatory events that lead to development of osteoarthritis

41883610087

CELL BASED THERAPIES FOR OA TREATMENT: THE SECRET OF SUCCESSFUL CARTILAGE REGENERATION IS HIDDEN IN THE STEM CELLS' ORIGIN.

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INTRODUCTION: Osteoarthritis (OA) is the most common arthropathy and a leading cause of pain and disabilities worldwide. Globally, OA affected more than 300 million people; however, despite its prevalence and importance, even economically, the comprehension of its molecular background is limited. Moreover, as OA is characterized by a complex molecular network, it is unlikely that targeting a single molecule would be sufficient to treat the disease. In this perspective, the idea of cell-based-therapies has gained much attention to possibly revert and stop the disease's progression. However, even though the effects exerted by stem cells on chondrocytes have been investigated, it's not clear yet if the stem cell's niche and their potency affect the quality of the tissue regeneration. For these reasons, the aim of this work was to investigate the effects exercised by different stem cell populations (multi- and pluripotent) on diseased chondrocytes, to identify the key molecules responsible for a successful articular cartilage (AC) regeneration.

METHODOLOGY: The hASCs (human adipose stem cells), SVF (stromal vascular fraction), hAECs (human amniotic epithelial stem cells) and the OA chondrocytes were isolated from tissue biopsies in accordance with the 1975 Declaration of Helsinki and the Ethics Committees of the research institutes involved. The stem cells (SCs) were isolated and kept in their specific growth media (GM) until further processing. After chondrocytes isolation,3D cultures were prepared (50 % chondrocytes + 50 % SCs), cultured at 37°C in humidified atmosphere (5% CO_2) and soaked by GM (DMEM high glucose, 10% FBS, pen/strep, amphotericin B, ascorbic acid 0.05 mM). After 7 and 21 days of culture, the pellets were sacrificed to extract the total RNA according to Chomczynski method. Then, the gene expression of healthy and hypertrophic articular cartilage markers was analysed with qRT-PCR, and the results were reported as relative gene expression (fold change -FC).

RESULTS: The qRT-PCR results indicated that the hASCs induced the best regenerative response in OA chondrocytes; indeed, while the AC markers were up-regulated those associated with fibrotic and hypertrophic phenotypes were down-regulated. The treatment with SVF instead, revealed an increase of runx2 and col10a1 expression, suggesting that the heterogeneous population of SVF might not be the more appropriate source of bioactive factors for AC regeneration. When OA chondrocytes were treated with AECs, the expression of transcription factors peaked earlier (7 d); however, the results don't display any clear trend; indeed, besides the up regulation of AC specific genes, there was an increase in the expression of hypertrophic markers. Nevertheless, these results reflect the plasticity of hAECs which, depending on the microenvironment, can finely modulate the components of their secretome, affecting the overall biological outcome.

CONCLUSIONS: To conclude, the results clearly indicated that the stem cell populations differ

in their ability to induce a high-quality AC. However, these encouraging results need further investigation with high-throughput techniques to map accurately the differently expressed molecules, that may reveal important hints for OA fighting.

keywords: Stem cells; osteoarthritis; cartilage regeneration

52354542088

CELL MORPHOLOGY AS A BIOLOGICAL FINGERPRINT FOR DESCRIBING CHONDROCYTE PHENOTYPE UNDER ACUTE AND CHRONIC IL-1B MEDIATED INFLAMMATION IN HEALTHY AND OSTEOARTHRITIC CHONDROCYTES

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Introduction: High IL-1β levels are often found in osteoarthritic (OA) joints. Moreover, IL-1β credibly induces post-traumatic OA. Since cell morphology is an important regulator of cell behavior, we investigated how acute vs. chronic IL-1β dynamically changes multifaceted aspects of cell morphology in healthy and OA chondrocytes in relation to phenotypic outcome.
Methods: Acute inflammation was mimicked by treating healthy bovine chondrocytes with IL-1β for 3 days, followed by media lacking IL-1β for 3 days. Chronic IL-1β was simulated by adding IL-1β to the media for 6 days. Unstimulated chondrocytes served as the control. An automated high-throughput method for quantitatively measuring a panel of shape descriptors and gene expression profiling (ddPCR) was used. Significant correlations and the correlation coefficient were calculated.

Results: Chronic IL-1β in human OA chondrocytes significantly decreased COL2A1, SOX9, and ACAN and increased IL-6 and IL-8 vs. control. At day 6 after acute inflammation, healthy bovine chondrocytes expressed significantly lower COL1A2 and higher IL-6, while chronic IL-1β significantly decreased COL2A1 and increased IL-6 and IL-8 vs. control and acutely-stimulated chondrocytes. IL-1β caused OA chondrocytes to become less wide, smaller, longer, slimmer, less round and more circular, consistent with a de-differentiated phenotype, while acute IL-1β caused healthy chondrocytes to increase in size, length, aspect ratio and become less round and solid. Chronic inflammation led to a decrease in area, length and aspect ratio, while the cells got more circular, round and solid. Significant correlations showed that both types of chondrocytes responded to IL-1β but behaved differently in their response. In OA chondrocytes, COL1A2 positively correlated with SOX9, while COL2A1 positively correlated with SOX9 and ACAN and negatively with IL-8. ACAN also negatively correlated with IL-8 expression. In OA chondrocytes, COL2A1, SOX9 and ACAN positively correlated with roundness, while SOX9 negatively correlated with the cell's major axis. The living cell number negatively correlated with OA grade, circularity and solidity. In healthy chondrocytes, there were positive correlations between COL1A2 and COL2A1 and IL-6 and IL-8, which was in contrast to OA chondrocytes. In healthy chondrocytes, COL1A2 also negatively correlated with IL-8, while COL2A1 negatively correlated with both IL-6 and IL-8. COL2A1 positively correlated with area, major axis and aspect ratio and negatively with circularity, roundness and solidity. Opposite correlations were found with IL-6 and IL-8 gene expression and the aforementioned cell descriptors.

Conclusion: IL-1β significantly alters chondrocyte morphology and the effects can be correlated to changes in chondrogenic and inflammatory gene expression. While IL-1β led to less of a de-differentiated cell shape in healthy chondrocytes vs. OA chondrocytes, importantly, IL-1β caused early morphological effects as well as functional effects in non-diseased and previously healthy chondrocytes suggesting that IL-1β could promote the "healthier" parts of the cartilage tissue to become diseased and enhance progression towards full OA. Quantitative cell morphometry may

be a useful biological fingerprint for describing chondrocyte phenotype under inflammatory attack and used to understand how inflammation or therapeutic targeting of inflammation regulates cell function and outcome.

keywords: osteoarthritis, inflammation, single cell morphology, early diagnosis

73296306655

EFFECTS OF LACTIC ACID ON SYNOVIAL FLUID

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Introduction: Synovial fluid is a viscous liquid found inside the joints of bones. This fluid helps in minimizing the bone to bone contact and facilitates movements of joints via lubrication. The key component of synovial fluid which gives its viscous property is hyaluronic acid (HA). Apart from HA it also consists of various proteins like albumins, gamma globulins etc. Synovial fluid has a unique viscoelastic property by which it protects the cartilage tissue from tearing down from joint movements. Alteration of this viscoelastic property leads to different arthritic conditions. There are different types of arthritic conditions starting from osteoarthritis, rheumatoid arthritis, gouty arthritis etc. It has been found that in every arthritic condition synovial fluid lactate level were different. Specially in case of rheumatoid arthritis, synovial lactate levels were found to be elevated. The presence of elevated lactic acid is might be due to from anaerobic respirations of some infectious bacteria. The presence of elevated lactic acid leads to decrease in pH conditions. This low pH environment might decrease the molecular weight of hyaluronic acid which eventually decreases the viscosity of hyaluronic acid. In order to know how lactic acid affects the synovial fluid viscoelasticity, microrheology as well as bulk rheology experiments were performed for hyaluronic acid and prepared model synovial fluid which consists of albumin and gamma globulins in addition to HA.

Materials and Methods: HA concentration was used 3.4 mg/ml prepared in 10mM phosphate buffer at pH 7.4. For model synovial fluid preparation, 3.4 mg/ml of HA, 10 mg/ml of bovine serum albumin (BSA) and 0.5mg/ml γ- Globulin were mixed. For the study of effects of Lactic acid, L-Lactic acid with different concentrations ranging from 1mg/ml to 5mg/ml were used. Microrheology as well as Bulk rheology experiments were done using Diffusing Wave Spectroscopy (DWS) and Anton Par MCR 301 Interfacial rheometer respectively.

Results and Discussions: In the microrheology experiment, there is a slight decrease in decay time of normalized intensity correlation function (ICF) of HA with increasing lactic acid concentrations. From Bulk rheology experiment, the viscosity of HA was also found to be reduced with Lactic acid concentrations. This might be due to decrease in chain length of HA. On the contrary, DWS of model SF, there is drastic change in ICF decay, where Lactic acid treatment significantly decreases the time for ICF decay. Further Bulk rheology experiment shows the notable decrease in viscosity of Lactic acid treated samples.

Conclusion: From microrheology as well as bulk rheology experiments it was established that Lactic acid treatment creates an environment where protein binds with HA and coprecipitates. This might be the reason behind the decreased synovial fluid viscosity in rheumatoid arthritis conditions.

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keywords: Albumin, Hyaluronic acid, Microrheology, Diffusing Wave Spectroscopy, Rheumatoid arthritis

PS65
Vascularization for Tissue Engineering and Regenerative Medicine

62825442939

ADDITIVE MANUFACTURE OF VASCULARISED SCAFFOLD FOR BONE TISSUE ENGINEERING

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Introduction
Most of the efforts in creating vascular structures using 3D printing have been concentrated on extrusion type of printing. However, mimicking vascular structures such as arterioles (100 µm diameter) is difficult due to the limitations in resolution that extrusion printing has. Inkjet 3D printing is a droplet-based type of printing that has resolution of 30 µm which can achieve printing features similar to arterioles. This project aims to fabricate vascularised bioactive scaffold using inkjet 3D printing, and biocompatible materials which consist of diacrylated PTMC-polyethylene glycol (PEG)-PTMC/gelMA/Polyethylene glycol diacrylated (PEGDA) as scaffolds matrix and water-soluble (WS) ink as sacrificial ink for vascular channel formation.
As an ink, gelatin methacrylate (gelMA) maintains excellent biocompatibility, solubility, and easy acquirement which is suitable for biomedical application1. Poly-trimethylene carbonate (PTMC) which has adjustable mechanical properties and non-acidic degradation produtcs2 can be used as a blend to improve mechanical stability. PTMC was modified into amphiphilic tri-block copolymer which is soluble in water so that the toxic organic solvent can be avoided.

Methodology
GelMA was synthesized in-house via precipitation method. The tri-block copolymer was synthesized following Ruiz et al3. Ink formulation was explored to find the optimum printability and crosslink ability using lithium phenyl-2,4,6-trimethyl-benzoyl phosphinate (LAP) as photoinitiator. For scaffold fabrication, Dimatix inkjet printer equipped with Samba cartridge was used. The samba cartridge consists of 12 nozzles with 2.4 pL drop volume allowing the high-resolution printing. The obtained scaffolds were then characterised by swelling test, mechanical test, and cells culture test using iMSC and HUVECs to analyse the bone tissue formation.

Results
The synthesized tri-block copolymer was a viscous liquid at room temperature and soluble in water. The 1H NMR results showed PEG, TMC, and acryloyl group signals without any remained monomer. Therefore, the synthesis process and purification process of diacrylated tri-block copolymer was successfully done. It was then used for ink formulation containing gelMA, triblock copolymer, and PEGDA with ultra-pure water as a solvent. Different concentration of each material was explored and analysed by liquid handler to find the optimum composition that is 1.5 wt.% gelMA, 10 wt.% copolymer, and 20 wt.% PEGDA700. The size of WS ink droplet is 48 ± 4 µm which offers small channel formation. After water immersion, the WS ink was completely removed and remained the vascular channel with the size of 109.00 ± 2.45 µm. The obtained gelMA/copolymer/PEGDA700 scaffold has better mechanical and adhesion properties compared to PEGDA700 scaffolds which allow better cells attachment.

Conclusions
GelMA, amphiphilic triblock copolymer, and the hybrid materials of those polymers which

dissolved in water were successfully prepared. It could minimize the use of an organic solvent which toxic to the cells and then enhance the biocompatibility. The vascularised scaffold was successfully fabricated using the ink formulation and the characterization showed that obtained scaffold has better properties for cells compared to PEGDA700 scaffolds.

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keywords: inkjet 3D-printing, vascularised scaffold, bone tissue engineering

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BEHAVIOR OF DUAL-CROSSLINKED GELATIN AND ITS POTENTIAL INFLUENCE ON VASCULARIZATION

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INTRODUCTION
Vascularization remains a critical factor for the success of various applications in the biomedical field. Insufficient blood vessel systems often limit the availability of replacement tissues via TERM.[1] Hence, there is a great interest in materials that potentially improve vascularization. The mechanical properties of hydrogels strongly influence cell behavior and organization. A promising approach for tunable hydrogel stiffness is dual-crosslinking, i.e., combining physical and chemical crosslinking by cooling and UV-irradiation.[2] This study investigated the influence of dual-crosslinked gelatin-methacryloyl (GM) on hydrogel material properties and HUVECs cell formation. Additionally, we employed mimicry of placenta extracellular matrix as a highly vascularized organ within the hydrogels to potentially improve vascularization and investigate the effect of hydrogel stiffness.

METHODOLOGY
Gelatin was synthesized with methacrylic anhydride, and formulations were prepared with varying macromer concentrations using lithium phenyl-2,4-6-trimethylbenzoylphosphinate (LAP) as photoinitiator in an aqueous solution. Human Placenta Substrate (hpS) was incorporated within the network (50 vol%) as a source of angiogenic factors. Crosslinking was performed either chemically with UV light at ~37 °C, or as dual-crosslinking (UV crosslinking of physically crosslinked GM at 4 °C). Gelation was monitored by photorheology, and in vitro analysis was performed using HUVECs.

RESULTS
The crosslinking mechanism significantly influences the stiffness of the gelatin-methacryloyl (GM) hydrogels. GM's dual-crosslinking increases hydrogel stiffness; however, double bond conversions do slightly decrease.[2] Hydrogel vascular network formation with HUVECs was observed when placenta-specific factors were incorporated, yet increased hydrogel stiffness resulted in confluency.

CONCLUSION
We suggest an explicit dependency between HUVEC behavior and hydrogel stiffness. Stiffer networks (due to higher macromer concentration or double-crosslinking) tend to confluence and less network formation. The crosslinking mechanism influences cell morbidity as well as behavior and should not be neglected, especially during the preparation process of GM hydrogels.

ACKNOWLEDGMENTS
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keywords: dual-crosslinking, hydrogels, vascularization,

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BIONIC PANCREAS - THE FIRST RESULTS OF FUNCTIONALITY OF 3D-BIOPRINTED BIONIC TISSUE MODEL TRANSPLANTATION WITH PANCREATIC ISLETS

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*Purpose: Tissue engineering is currently on advanced stage of development which gives a possibilities for novel strategy of personal treatment of type 1 diabetes. AIM: In the following study, a bioink based on ECM derived from decellularization of porcine pancreas was applied for 3D bioprinting.

*Methods: The SCID (n=60) and BALB (n=20) mice were used as a model for in vivo study. Porcine islets mixed with bioink were printed on extrusion printer and transplanted on studied animals. Effectiveness of transplanted petals with regard of their insulin secretion was evaluated based on glucose and c-peptide concentration in blood samples of studied animals. Thus, animals were divided into three groups: mice with transplanted islet-laden petals, mice with transplanted islets into kidney capsule and untreated mice. Examination of studied parameters took place at four time points during the experiment, at the beginning and on day 7th , 14th and 28th day of experiment.

*Results: Group with transplanted petals from day 7th expressed lower mean fasting glucose concentration while compared with untreated group (129 mg/dl, 119 mg/dl, 118 mg/dl vs. 140 mg/dl, 139 mg/dl, 140 mg/dl respectively in 7th, 14th and 28th day post-transplantation; $p<0.001$). Post-surgery transverse section of petals revealed that connective tissue of studied animals surrounded and stabilized transplanted petals. Fibroblasts infiltration over time resulted in the process of new blood vessels formation within the petals. Hence, presented in the study bioink provides a favorable conditions for islets functionality. The bioprinted construct was stable over time. Furthermore, no pathological conditions of studied animals were observed which indicates that bioprinted petals were biocompatible.

*Conclusions: Bionic flake transplantation lowered glucose levels significantly.

keywords: ECM, T1D, Bioprinting, Bioink

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BUILDING VASCULAR MUSCLE TISSUE FROM THE BOTTOM-UP

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Introduction
The engineering of functional macrotissues, such as muscle tissue, is challenging due to the limited degree of diffusion, resulting in poor cell survival rates. To overcome this problem, the incorporation of a (mature) vascularized network is important. Therefore, a possible strategy is conducting bottom-up tissue engineering by generating vascularized tissue-specific spheroids that can serve as tissue building blocks. The use of these microtissue building blocks is advantageous compared to single cells because of their high cell density, improved cell survival, microarchitecture and accelerated ECM production. When spheroids are combined with a suitable hydrogel, a bioink can be obtained. This bioink can be used for the creation of more complex macrotissues via 3D bioprinting.

Methodology
Myogenic vascularized spheroids were generated by seeding different ratios of human myoblasts, endothelial cells and mesenchymal stem cells (1,0x106 cells) in an in-house developed microchip system with 400 µm pores. After 4 days of cultivation in medium specialized for endothelial cell growth (EGM-2), spheroids were exposed to myogenic differentiation medium consisting of DMEM:glutamax supplemented with 50 µg/ml gentamicin, 50 µg/ml bovine serum albumin, 10 µg/ml human insulin and 10-10 g/ml human epidermal growth factor. Analysis was performed after 4, 8 and 12 days of chip culture. Immunohistochemical staining for tropomyosin and CD31, as well as, histological and live/dead analysis was conducted. With this, the morphology, ECM production, differentiation and spheroid viability was evaluated.

Results
Vascularized myogenic spheroids could be obtained containing several ratios of cells. The spheroids remained viable over time. (Immuno)histochemical analysis showed the presence of tissue-specific ECM components and the formation of microvascular networks.

Conclusion
During this study it was demonstrated that vascularized myogenic spheroids can be obtained to use as a part of a printable bioink. Future work will investigate the fusion capacities of the spheroids, as well as, the alignment of fibers and optimal spheroid maturation stage for encapsulation in several types of hydrogels.

keywords: 3D cell culture, spheroids, vascularity, myogenic tissue, 3D bioprinting

31412701455

COMPARING THE THERAPEUTIC POTENTIAL BETWEEN AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS AND ALLOGENIC UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS IN CRITICAL LIMB ISCHEMIA: A PILOT STUDY

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Introduction
Critical limb ischemia (CLI) represents the final stage of peripheral arterial disease. Approximately one-third of patients with CLI are not candidates for conventional surgical treatment. Autologous bone marrow mononuclear cells (BM-MNC) and allogenic umbilical cord-derived mesenchymal stem cells (allo-UC-MSCs) have emerged as a promising therapeutic approach for this condition.

In the present work, we compare BM-MNC vs. UC-MSC, evaluating its safety and efficacy profile in comparison with the placebo group in patients with CLI.

Methodology
Fifteen injections of: (i) BM-MNC (7.197x106 ± 2.984 x106 cells/mL each with 2% of autologous serum), (ii) allo-UC-MSCs (1.333 x106 cells/mL each with 5% of human serum albumin serum) or (iii) vehicle (1 mL saline solution with 2% of autologous serum) were administered into the periadventitial arteries. Twenty-four patients in the most severe stages of the disease (category 4 or 5 in Rutherford's classification and transcutaneous oxygen pressure (tcpO2) below 30 mm Hg were randomized to receive: (i) BM-MNC (n=7), (ii) allo-UC-MSCs (n=7) or (iii) placebo (n=10). The follow-up visits were at months 1, 3, 6 and 12, in order to evaluate the following parameters: (i) Rutherford classification, (ii) tcpO2, (iii) percentage of wound closure, (iv) pain, (v) pain-free walking distance, (vi) revascularization and loss of the limb during follow-up and (vii) the clinical outcome scale (EQ-5D questionnaire).

Results
No adverse events were reported. Patients with CLI that received BM-MNC and allo-UC-MSCs presented an improvement in Rutherford classification, a significant increase in tcpO2 values, clinical changes in the lesion size or its closure in a shorter time, a more noticeable decrease in the pain score, and an increase in the pain-free walking distance from the first-month post-treatment, in comparison with the control group. In addition, the participants treated with BM-MNC and allo-UC-MSCs kept their limbs during the follow-up period and surgery was not required, unlike the control group participants, who had a marked increase in the amputation of the affected limb and even two participants required a revascularization process.

Conclusion
Our cumulative results suggest that BM-MNC and allo-UC-MSCs in patients with CLI lead to significant clinical changes, being more noticeable in a shorter time with allo-UC-MSCs, unlike the control group where the participants increased their classification to a more severe stage of the disease: Rutherford 6. Thus, our pilot is clinically relevant as it highlights the possible use of BM-MNC or allo-UC-MSCs as a novel therapeutic approach to treat CLI, which could be part of its comprehensive management.

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keywords: Lower limb ischemia, autologous bone marrow mononuclear cells, allogenic umbilical cord-derived mesenchymal stem cells and cell therapy

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CONTROLLED METABOLITE RELEASE FOR TISSUE SURVIVAL AND INTEGRATION IN ANOXIA

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Introduction: In the last decade many advancements have been made in engineering tissues that function as replacement for malfunctioning or damaged tissues and organs(1–3). However, current methods are limited to either thin tissues such as skin and bladder(4), or small sized tissues used in small rodent models(2). Scaling these tissues up towards clinical relevant sizes currently remains a major unresolved challenge in tissue engineering. Specifically, increasing tissue size associates with progressively severe oxygen and nutrient diffusion limitations, which causes cellular necrosis and ultimately implant failure(1–3). Currently, lack of oxygen is considered the main factor leading to necrotic core formation. As a result a variety of solutions to oxygenate engineered tissues have been developed, often using peroxides to generate oxygen in situ (5, 6). However, the effect of nutrient availability under diffusion limit-induced anoxic conditions has remained largely unstudied. Therefore, we have investigated the effect of various metabolites on cell survival under anoxic conditions.

Methodology: Human mesenchymal stem cells (hMSCs) were seeded at a density of 4000 cells/, and cultured without media change for seven days in serum-free and metabolite-free chemically defined media, except for the single type of metabolite as the experimental condition. These metabolites included various sugars, lipids, amino acids and vitamins. Cell viability and functionality were determined using standard cell culture techniques. The glucose release system was engineered by casting polycaprolactone-glucose melt into discs, which were then embedded into gelatin-methacryloyl hydrogels containing 3x hMSCs/ml. Glucose release was determined using a glucose oxidase-DAB assay.

Results: Sugars consistently allowed for high percentage of hMSC viability, while other conditions resulted in massive cell death. This confirms that nutrient (e.g., sugar) depravation rather than anoxia induces cell death. Glucose was then chosen as the model sugar and was used in a metabolite release system, which was able to maintain viability and metabolic activity in 3D cell laden hydrogel constructs. Importantly, the high cell viability combined the anoxic environment resulted secretion of high amounts of pro-angiogenic factors, such as VEGF. This resulted in the vascularization and therefore integration of a subcutaneously implanted tissue. Moreover, unlike commonly explored oxygen generating peroxides, glucose achieved this survival and vascularization in the absence of cytotoxic radical formation.

Conclusion: Metabolism supporting biomaterials are introduced as a novel method to support implant survival and realize functional integration within a host. Specifically, a glucose is identified as a key metabolite maintain cell survival under anoxic conditions, and controlled release of glucose allows for the survival and vascularization of living implants.

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keywords: anoxia, human mesenchymal stem cells, metabolites, angiogenesis, GelMA

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FREESTANDING COLLAGEN HOLLOW FILAMENTS - A TOOL FOR VASCULARISATION OF IN VITRO 3D TISSUE MODELS

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Introduction
Microfluidic cultivation platforms significantly improve the supply of tissue models in vitro. However, a key challenge is the sufficient distribution of oxygen and nutrients within a 3D construct. Here we demonstrate the use of collagen hollow filaments as a functional unit for the generation of a vascular structure in tissue engineering applications.

Methodology
The hollow filaments with a diameter < 1 mm were fabricated by direct extrusion of a collagen fibre suspension through a core-shell nozzle. The filaments were cross-linked, freeze-dried and mechanically characterised. The swelling behavior of the tubes and the permeability of the tube wall for nutrients and oxygen were analysed more in detail. Human endothelial cells (ECs) were seeded on the inner surface of the tubes and cultured under perfused conditions in a microfluidic circulation system. The shrinkage of the tube during cell cultivation was monitored also.

Results
The fibres are mechanically stable and permeable for oxygen and proteins. ECs growing on the inner surface showed typical features of a well-formed endothelium including VE-cadherin expression, cellular response to flow and secretion of extracellular matrix proteins. Cell growth was analysed over a period of 21 days. During this time, shrinkage of the cell-laden hollow tube was < 10%.

Conclusion
We were able to confirm that the collagen hollow filaments support the formation of a living vascular tissue over a long period. The fibres enable the delivery of nutrients and oxygen to a surrounding compartment. Therefore, the collagen hollow filaments could be used as a template for the fabrication of prevascularised tissue engineering constructs.

keywords: Collagen, Vascularisation, Blood vessel, Perfusion, In vitro

31412757519

GELATIN-PVA MICROSPHERES FOR DUAL GROWTH FACTOR DELIVERY TO GUIDE VASCULARIZED BONE FORMATION

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Introduction
Tissue engineering employs a variety of different cell friendly platforms capable of supporting and enhancing cell growth and proliferation [1]. Bone and endothelial tissue interaction is closely connected since bone formation depends on vascularization [2]. This work reports on the fabrication of microspheres as biocompatible carriers for the controlled delivery of the growth factors bone morphogenetic protein 2 (BMP-2) and vascular endothelial growth factor (VEGF), responsible for bone and endothelial tissue formation respectively. Polyvinyl alcohol (PVA) and gelatin were blended in different compositions to determine the concentration with the optimal biocompatibility, degradation rate, and protein release using appropriate cell types.

Methodology
The microspheres were fabricated following the emulsion cross-linking technique. PVA solutions in deionized water were prepared at final concentrations of 5, 7 and 10% w/v. Gelatin (10% w/v) was dissolved in the aqueous PVA solutions under stirring. The mixtures were added dropwise through a syringe to sunflower oil to create a water-in-oil emulsion. 1 ml of 50% v/v glutaraldehyde, and then 30 ml of pure acetone were added. The microspheres were collected through centrifugation at 3500 rpm, washed with acetone and lyophilized. Their morphology was investigated by means of scanning electron microscopy (SEM). Four samples with different % w/v PVA concentrations (0, 5, 7, and 10% w/v) were used to encapsulate bovine serum albumin (BSA) as a model protein. The microspheres were evaluated for % erosion, loading capacity (LC%) and encapsulation efficiency (EE%). The biocompatibility of the different PVA-gelatin microspheres concentrations (0.125, 0.25, 0.5, 1, and 2 mg/ml) was assessed using L929 fibroblasts. Ongoing experiments with BMP-2- and VEGF-loaded microspheres are in progress.

Results
The fabricated microspheres have a diameter ranging from 20-200 μm depending on the concentration of PVA. Microsphere size distribution measurements reveal a positive correlation between PVA concentration and microsphere diameter, which is expected to affect the rate of drug release. The EE% ranged from 80% to 96%, and the % LC from 129% to 155%. Protein release studies depict that the initial burst release occurs during the first 24 h, followed by a steady release rate of protein during a 350 h incubation period. The 0%PVA composition demonstrated the fastest release kinetics with almost 80% release of total encapsulated protein during the first 24 h. 5% and 7% PVA microspheres displayed comparable release kinetics with values of 20% after 24 h and 35% during the first two weeks. An increase of PVA concentration up to 10% led to an even slower protein release profile. The investigated concentrations from 0.5 mg/ml and lower present similar or higher cell viability values compared to the TCPS control.

Conclusion
The PVA-gelatin microspheres are highly biocompatible, demonstrating a low initial burst release and a prolonged cumulative release up to 14 days, thus they function as effective growth factor carriers.

Acknowledgement
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keywords: bone morphogenetic protein 2, vascular endothelial growth factor, vascularisation, bone tissue repair

94238156808

NOVEL WHEY PROTEIN ISOLATE-BASED HIGHLY POROUS SCAFFOLDS MODIFIED WITH CU- AND CO-DOPED BIOACTIVE GLASSES

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The search for new or alternative materials for use in bone tissue engineering (BTE) is one of the main challenges. Whey protein isolate (WPI) as a by-product of cheese manufacturing is inexpensive and widely available. Heat treatment of an aqueous solution of WPI above 60 °C results in the formation of a three-dimensional hydrogel network without the use of additional chemical cross-linking agents. A group of widely studied biomaterials for BTE includes bioactive glasses (BGs). Attractiveness of BGs lies in their ability to bond to living bone (bioactivity). Furthermore, their ionic dissolution products have been shown to stimulate bone formation via activating osteogenic genes (osteoinductivity). BGs can be used as effective carriers for inorganic therapeutic ions. Copper (Cu) and cobalt (Co) ions are known to induce new blood-vessel formation (angiogenesis). This has recently attracted particular attention because angiogenesis, besides osteogenesis, is essential for complete bone tissue regeneration.
In this work, for the first time, a material derived from food industry waste – WPI - and a material commonly used in bone regeneration – BGs - were combined to obtain novel composite biomaterials with potential applications in BTE. Additionally, to obtain pro-angiogenic properties, BGs doped with Cu2+ and Co2+ ions were used. Simple method based on the gas foaming (NH4HCO3) and autoclaving was applied to produce highly porous WPI/BG scaffolds. Materials were evaluated in terms of microstructure using SEM, high-resolution X-Ray microtomography and mercury intrusion porosimetry. In vitro bioactivity was evaluated by incubation of materials in simulated body fluid (SBF). The samples after incubation were analysed using ATR-FTIR and SEM/EDX methods. The changes in Ca, P, Si, Cu, and Co concentration in the SBF were monitored using an ICP-OES technique.
Using a simple gas foaming method, ready-to-use (sterile), bioactive scaffolds with high porosity (above 70%), fully connected pore networks, and pore size suitable for BTE applications (80–350 μm) were obtained. Thanks to the use of Cu2+ and Co2+-doped sol-gel BGs, the scaffolds showed additional functionalities - they are able to develop bioactive calcium phosphate layer on their surfaces and to gradually release therapeutic ions. Porous WPI/BG composites showed great potential for the use as novel bone substitutes, however they require further studies, especially analysis of degradation kinetics and biological properties.
This work was supported by the National Science Centre, Poland Grant No. 2019/32/C/ST5/00386 (MD).

keywords: scaffold, whey protein isolate, bioactive glass, therapeutic ions

94238140266

SIMPLE GENERATION OF PERFUSABLE MICROVASCULAR NETWORKS IN 3D TISSUE MODELS VIA SACRIFICIAL POLY(2-OXAZOLINE) SCAFFOLDS

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Functional 3D tissue models are a potentially valuable tool to provide more transferable test results regarding basic research, drug development or other applications than classic 2D cell culture. The biggest bottleneck in the generation of functional 3D tissue models is the incorporation of a reproducible and perfusable vascularization, which on the one side mimics the in vivo hierarchy accurately, and on the other side keeps the tissue alive. Furthermore, perfusable vascularization provides the possibility to enhance the height of the tissue model above a threshold (usually 1-2 mm), where cells normally die due to lack of nutrients and oxygen. An interesting approach to generate microchannel networks in 3D tissue models is the utilization of sacrificial scaffolds. These scaffolds are prefabricated and can be introduced into cytocompatible hydrogels and, after gelation of the hydrogel, dissolved to generate channels. However, current methods are based on using sacrificial carbohydrates, which directly dissolve upon contact with aqueous environment. Thus, to create stable channels within a hydrogel system, these scaffolds require laborious postfabrication processing (e.g., coatings) to prevent their early dissolution during the hydrogel gelation process.

In this work, we introduce biocompatible poly(2-oxazoline)s as material for sacrificial scaffolds. These polymers are thermoresponsive in aqueous solutions, meaning that they dissolve on demand by temperature reduction, a very cytocompatible stimulus. This further leads to an increased dissolution timeframe without the need of any postfabrication processing and enables the utilization of many currently used hydrogels for biofabrication (e.g., methacrylated gelatin or collagen).

Interestingly, poly(2-oxazoline)s can be fabricated via modern additive manufacturing technologies like Melt Electrowriting and Freeform Printing to generate microfiber networks in 2D and 3D. This enables the generation of interconnected channel networks with bifurcations, resembling the natural vascularization. Furthermore, the characteristics of the polymer allow the prefixation of the scaffolds in specialized perfusion chambers. By simple addition of a cell-laden hydrogel, these perfusion chambers mediate the generation of microchannel-networks and their leakage-free connection to media-reservoirs. Furthermore, this allows the direct cultivation and endothelialization of the perfusable tissue construct in the perfusion chamber and its connection to perfusion devices.

Taken together, the utilization of poly(2-oxazoline)s as sacrificial scaffolds is a reliable technology,

which enables the simple generation and cultivation of perfusable vascularized tissue constructs.

keywords: Perfusion, Vascularization, Thermoresponsive Polymers, Biofabrication

31412728539

TISSUE ENGINEERING USING VASCULAR ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELL DERIVED ENDOTHELIAL CELLS AND MURAL CELL PHENOTYPES

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INTRODUCTION: Regenerating large tissues requires an intimate supply of the host vascular network, a slow process leading to low viability of the regenerating cells. A solution to this obstacle is the generation of prevascularized tissue engineered constructs. Since Endothelial (ECs) and Mural (MCs) cells, such as smooth muscle cells (SMCs), and pericytes (PCs), are the cellular components of blood vessels and their interactions are crucial for neovascularization, both cell types and their arrangement into correct spatial organization are required in order to rescue tissue engineered constructs from critical ischemia and to form a functional vascular network in vivo. Based on this context and in order to overcome the limitations concerning the isolation and expansion of human SMCs, we developed a protocol to differentiate human pluripotent stem cells (hPSCs) to SMCs. In addition, we generated ECs from the differentiation of hPSCS (hPSC-ECs). hPSCs-SMCs and ECs (hPSC-ECs or primary ECs) were then used to generate 3D vascular organoids which rapidly give rise to a complex three-dimensional vascular network.

METHODOLOGY: We developed an in vitro approach to induce the differentiation of hPSCs (human embryonic stem cells and human induced pluripotent stem cells) to defined SMC populations (contractile and synthetic hPSC-SMCs) using feeder-free and low serum conditions. hPSC-SMCs phenotypes and hPSC-ECs were extensively characterized concerning their phenotype and function. Subsequently, using a methylcellulose-based hydrogel system, we generated spheroids consisting of 1,000 cells (ECs and hPSC-SMC) (vascular organoids). Vascular organoids were extensively characterized regarding their phenotype, cell-cell interactions and their ability to form a three-dimensional capillary network in vitro. Finally, we investigated the vascularization potential of these vascular organoids, when embedded in hydrogels composed of defined extracellular components (collagen/fibrinogen/fibronectin) that can be used as scaffolds in tissue engineering applications.

RESULTS: hPSC-SMCs were phenotypically and functionally stable for at least 8 passages and had the ability to stabilize vessel formation and inhibit network regression, when co-cultured with hPSC-ECs in vitro. Furthermore, hPSC-EC/hPSC-SMCs vascular organoids served as focal starting points for the sprouting of capillary-like structures in vitro, using defined matrices of extracellular matrix components.

CONCLUSIONS: We developed a robust method for the generation of defined hSMCs phenotypes from hPSCs. In addition, we differentiated hPSCs to ECs. Fabrication of hECs/hPSC-SMCs vascular organoids embedded in chemically defined matrices is a significant step forward in tissue engineering and regenerative medicine.

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keywords: induced pluripotent stem cells, smooth muscle cells, tissue engineering, vascular organoids, vascularization

41883650679

TOWARDS BIONIC ORGANS: BIOCOMPATIBILITY OF NEWLY DEVELOPED PORCINE DECM-BASED HYDROGELS

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There is a growing interest in fabrication of bioinks which on one hand biocompatible and on the other hand possess mechanical properties which would allow to fabricate stable constructs capable to survive for a long time after transplantation. Although choosing appropriate material is essential for bioprinting, there is however, another, equally important issue extensively studied nowadays – inclusion of vasculature system within fabricated scaffolds. Thus, in the following study we designed artificial channel bioprinted of ECM based so called “bioink A” to investigate if essential for neovascularization endothelial cells (HUVEC) and fibroblasts (aHDF) with proportions of 1:2 (8 mln/mL in total), would adhere to the material. Additionally, a media flow through such perfused channel was set to stimulate cell adhesion and proliferation. Fiber of bioink A which formed vault of a channel was printed either parallelly or perpendicularly to the direction of media flow. Two ways of seeding cells was tested. Channel was either printed with cell-laden so called “bioink B” or cells were delivered to the channel directly, with pipetting. In each seeding variant, a total of 4·105 cells per channel were used. After 2, 5, 8 or 24h of incubation, media flow was applied. After 8 days of experimental trial for each time variant, the channels were stored in formaldehyde and immunohistochemical staining was made to investigate the presence of cells on channel walls and vault. Cells adhered for both ways of fiber arrangement, however parallel bioprint with 5h of incubation and direct cell seeding resulted in better adhesion efficiency. After 5h of incubation, before flow was set, 2.1·105 cells stayed in channel with 75% viability. The quantity of cells did not decreased over time, until the end of experiment. Hematoxylin & Eosin staining showed that after 8 days cells were uniformly distributed across vault of a channel. Our study clearly shows that cells which promote neovascularization adhere efficiently to pancreatic, ECM based bioink. It proves that bioink A of pancreatic origin can be used also for other than pancreatic cells type. Presented in this research bioink B can be used for other studies as vector for cell seeding.

keywords: vessels, HUVEC, fibroblasts, 3D bioprinting, bioink, extracellular matrix, bionic organ, extrusion

20941834648

UNITING SPHEROIDS, HYDROGELS AND HYPOXIA TO PUSH THE MATURATION OF 3D PRINTED VASCULARIZED TISSUES

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Introduction
3D bioprinting is ideally positioned to promote the (vascular) complexity of in vitro engineered tissues. However, the resolution of extrusion-based printing, the most widespread bioprinting technique, is too limited to create microstructural features such as capillaries. Therefore, we have previously developed printable vascularized spheroids (Ø≈100-130 µm) by seeding human umbilical vein endothelial cells (ECs), human foreskin fibroblasts and adipose-tissue derived mesenchymal stem cells (MSCs) onto agarose microwell chips. These spheroids remain stable and viable over at least 10 days and the ECs organize into a capillary-like network1.
The spheroids can be printed in a methacrylamide-modified gelatin (GelMA) hydrogel and extensively grow out in the gel. Sprouts of individual spheroids inosculate and the spheroids fuse, but only when they are placed in close proximity2. Unfortunately, the encapsulation density is currently too low to obtain extensive fusion of all spheroids into one tissue. Therefore, our current efforts focus on the optimization of the printing protocol and the maturation of the constructs post-printing.

Methodology
We aim to advance the spheroid outgrowth and fusion by lowering the hydrogel concentration and enhancing the encapsulation density. We print 6-layered grid constructs with a 0°/90° lay-down pattern or macrovascular cylindrical constructs with an inner diameter of 1 mm.
Since hypoxia is a known stimulator of angiogenesis, we are also assessing the impact of low-oxygen culture on non-printed and printed spheroids.
Spheroids are analyzed via light, fluorescence and confocal microscopy. Immunohistochemistry/qRT-PCR is applied to detect growth factors as vascular endothelial growth factor and vascular elements as VE-cadherin, collagen type IV, laminin and EC markers as CD31.
In addition, we are innovating our protocol using human induced pluripotent stem cells (hiPSCs). We are currently differentiating hiPSCs, transfected with the endothelial transcription factor ETV2, to ECs3. These cells will be combined with hiPSC-derived MSCs to create hiPSC-derived vascularized spheroids.

Results
We were able to reduce the GelMA concentration from 10 to 8 w/v% while maintaining a stable construct integrity. This reduced concentration allowed us to double our original encapsulation density of 22 920 spheroids/ml gel. The spheroid outgrowth and fusion was strongly increased compared to our previous results, but it was not complete yet so further optimizations are required.
Preliminary data indicate that hypoxia slightly increases the vascularization in non-encapsulated spheroids. Data on the influence on printed spheroids will be gathered in the coming months.
Lastly, we could successfully differentiate hiPSCs to ECs and we will combine them with hiPSC-derived MSCs in a spheroid culture in the following weeks.

Conclusion
Spheroids form versatile building blocks for the 3D bioprinting of vascularized tissues. Lowering

the hydrogel concentration, enhancing the encapsulation density and the implementation of hypoxia show to promote spheroid vascularization and fusion. Future experiments will further optimize and synergize these strategies to engineer one whole microvascularized tissue and answer the need for novel vascularization approaches in tissue engineering.

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keywords: spheroids, vascularization, 3D bioprinting, hydrogels, hypoxia

83767217105

VASCULARIZATION OF FULL-THICKNESS SKIN EQUIVALENTS

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Introduction
Anatomically-correct models of human skin are highly desired by both biomedical sciences and industry. Organotypic skin cultures, termed full-thickness skin equivalents, including fibroblasts and keratinocytes, thus forming dermis and epidermis are in use. However, a more detailed model of the skin is difficult to set up due to its cellular heterogeneity, complex vascular anatomy, and intricate interaction between the substructures. Therefore, the next generation of in vitro skin models needs to provide an accurate depiction of homeostatic, aging, or injured skin that capture the skin's cellular heterogeneity and complex anatomy. Since current full-thickness skin equivalents lack functional vasculature, circulation, perfusion or inclusion of the immune system is not possible. As a solution, we here engineered a vascularized 3D full-thickness in vitro skin model (vascuSKINs) including epidermis, dermis, and vasculature by combining vascular self-assembly and full-thickness skin equivalent culture.

Methodology
Human dermal fibroblasts, endothelial cells, and adipose-derived stromal cells were embedded in fibrin-collagen co-gels before seeding of keratinocytes and epidermal differentiation at air-liquid interface culture for 14 days. Skin equivalents were fixed in 4% buffered formalin, embedded in paraffin, sectioned, and stained using immunofluorescence for histological analysis.

Results
In order to establish the vascuSKINs, we first investigated the suitability of fibrin-collagen co-gels as an extracellular matrix to support epidermal differentiation and microvascular self-assembly. In contrast to collagen-only hydrogels, the fibrin-collagen co-gels indeed supported vascularization and showed a limited contraction, thus enabling the formation and persistence of open lumina. In addition, histological analysis showed no differences in epidermal stratification of the collagen-fibrin co-gels compared to traditional collagen hydrogels: the epidermis stained positive for keratin 14, keratin 10, and loricrin, suggesting that it well supports keratinocyte differentiation and stratification. Importantly, endothelial cells embedded into the dermis formed a continuous, lumenized microvasculature as visualized by CD31 staining.

Conclusion
In summary, these results show the successful establishment of full-thickness vascularized skin equivalents using collagen-fibrin co-gels and human cells. Furthermore, our model resembles skin by including a dermis and an epidermis that consists of the essential cell types associated with wound healing. The next step of our project is to establish our vascuSKINs as models of skin injury and regeneration.

keywords: 3D model, hydrogel, skin equivalent, vascularization

PS67
We've got your back: the challenges and success of advanced regenerative treatments for intervertebral disc regeneration

62825462649

3D BIOPRINTING WHOLE INTERVERTEBRAL DISCS TO INFORM REGENERATIVE THERAPIES

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INTRODUCTION
Back pain is one of the leading causes of disability, with an estimated 540 million sufferers worldwide. Degeneration of the intervertebral discs (IVDs) is implicated in 40% of cases; however, treatment remains limited and regenerative therapies are becoming essential. 3D bioprinting provides a powerful tool to create biomimetic tissue analogues with properties closely matched to complex tissues. This study therefore attempts to use shear-thinning alginate-collagen hydrogels to replicate the complex mechanical, biochemical, and structural cues present within the IVD by 3D bioprinting a range of models for the developing, healthy, and degenerating IVD. It is hypothesised that by varying bioprinted hydrogel stiffness, incorporating important extracellular matrix components such as laminin, and by varying the IVD models' regionally specific macro- and micro-architecture, healthier disc cell phenotypes can be encouraged and the results used to inform regenerative strategies.

METHODOLOGY
Immortalised human cells from the IVD's central gelatinous nucleus pulposus (NP) and surrounding fibrous annulus fibrosus (AF) regions were suspended in blended alginate-collagen hydrogels using a protocol previously developed within the lab group1. Cell-laden hydrogels were then bioprinted using Suspended Layer Additive Manufacturing (SLAM), a novel technique appropriate for bioprinting low-stiffness (<~100kPa) materials. IVD models were designed using BioCAD and BioCAM, based on patient data. The mechanical properties of the bioprinted constructs were then characterised using rheology and compressive testing. Live/Dead staining was used to optimise bioprinting settings. Cells were tagged with cell-permanent tracker dyes and monitored for a period of 28 days using stereo fluorescence microscopy and confocal laser scanning microscopy. Cell phenotype was assessed using qPCR, for a panel of genes previously defined by the research group2, whilst tissue development was examined using immunostaining, for a range of key healthy and degenerate IVD markers.

RESULTS
A range of physiologically relevant stiffnesses and regionally specific stiffness gradients have been achieved within the bioprinted IVD models. All models supported high cell viability. Cell tracker studies indicated that the NP, AF and NP-AF interface regions were maintained over a culture period of at least 28 days. Immunostaining revealed substantial production of HA and ACAN, whilst qPCR demonstrated phenotypic responses to changes in stiffness and gel composition. The introduction of laminin into the hydrogels was shown to encourage NP cell clustering.

CONCLUSION
Using SLAM, soft hydrogels have been patterned to replicate an IVD with regionally specific NP-like, AF-like and interface-like regions, containing both human NP and AF cells. Softer and stiffer variants of the IVD can now be created to deliver 'healthy' and 'degenerate' IVD models based on patient data. The discovery that laminin encourages clustering is a particularly interesting result that must be investigated further when applied to a model of the foetal IVD, since laminin

is especially present at this early stage. The introduction of growth factors (TGFβ, GDF-5/6) is also of particular interest, since bioprinted gel composition has been shown to influence NP cell phenotype using both qPCR and immunostaining. The platforms developed will ultimately be applied to optimise protocols for IVD regeneration using primary NP cells and mesenchymal stem cells.

keywords: Bioprinting IVD regenerative hydrogels

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ASSESSING THE CLINICAL RELEVANCE OF PRE-CLINICAL MODELS THROUGH INVESTIGATING THEIR NUTRIENT MICROENVIRONMENT AND REGENERATION CAPACITY

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Despite exciting advances in regenerative medicine, cell-based strategies for treating degenerative disc disease remain in their infancy. After demonstrating safety and efficacy in animal studies, many of these therapies enter a critical period between preclinical validation and clinical evaluation, where they do not appear to work to the same extent in humans. It is commonly believed that the harsh nutrient microenvironment and "hostile" nature of the degenerating intervertebral disc is linked to high failure of prospective studies [1]–[3]. Therefore, in this work we aim to investigate the species-specific nutrient microenvironment and regenerative capacity of two commonly used animal models in attempts to ascertain their scientific merit for successful clinical translation.

In-silico models have been developed using COMSOL Multiphysics to predict the local nutrient microenvironment of a small rat tail and large goat lumbar pre-clinical model, using methods published previously [4]. These models are reinforced by experimentally determined input parameters such as species-specific metabolically active cell densities, geometries, and rates of metabolism. An active cell density is determined by using MTT and DAPI counterstain. Metabolic rates are determined for disc cells in a 3-D spheroid culturing using seahorse flux analyser. In addition to the nutrient microenvironment, we developed a GAG regeneration models for rat and goat, as seen previously for human [5], [6], using experimentally determined species-specific GAG synthesis rates.

This study provides a guide for designing pre-clinical animal models through understanding differing nutrient microenvironments within intervertebral discs of different size scale. By comparing these animal models to the degenerated human condition, we can demonstrate the predicted rate of regeneration and the period needed for significant extracellular matrix repair, to assess the efficacy of rat and goat studies for successful clinical translation of cell-based therapies.

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keywords: in-silico models, nutrient microenvironment, regeneration

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BIOPRINTED INTERVERTEBRAL DISC: IN VITRO EVALUATION OF A COLLAGEN/HYALURONIC ACID BIOINK WITH OVINE DISC CELLS

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Introduction. Intervertebral disc (IVD) degeneration is one of the main cause of low back pain (LBP), affecting over 600 million individuals worldwide. Mechanisms associated with the degeneration remain poorly characterized, in part due to the lack of adequate IVD models. Reproducing in vitro the complex structure of an IVD, with its gelatinous core Nucleus Pulposus (NP) surrounded by the anisotropic lamellae of the Annulus Fibrosus (AF), represents a challenge for tissue engineers. To develop a bioprinted IVD model, we selected collagen type I (Col) and tyramine-conjugated Hyaluronic Acid (THA) with different ratios, according to the native composition of the IVD, and we evaluated the interactions of ovine NP and AF cells with these bioinks.

Methodology. Col 0.4%-THA 0.6% (ratio 1:1.5) and Col 0.4%-THA 0.1% (ratio 1:0.25) hydrogels were selected to mimic the NP and AF tissues, respectively. To determine the effect of THA concentration on the stiffness of the hydrogels, Young's modull were measured with a MicroTester equipment (CellScale). NP and AF cells were harvested from healthy discs of lambs (age 6 months), in collaboration with the Nantes ONIRIS veterinary school. We encapsulated 1 to 6 million of these cells per mL (Col-THA 1:1.5 for NP cells and Col-THA 1:0.25 for AF cells). To characterize the interaction between the cells and the bioinks, cell viability was assessed with a Live/Dead assay and cell morphology was visualized by cytoskeletal actin labeling with fluorescent phalloidin- Alexa Fluor 647 (up to 28 days). Cell proliferation was assessed by a DNA assay (PicoGreen assay) and by a nucleoside incorporation assay during the cell cycle (EdU assay).

Results. The two hydrogels exhibited different mechanical properties with a Young's modulus of 2.0 kPa and 0.6 kPa for the 1:1.5 and 1:0.25 ratios, respectively. In addition, an increase in THA concentration increased the stiffness of the bio-inks. NP and AF cells encapsulated in both formulations remain alive for at least 28 days, confirming that the bioinks are not cytotoxic. While actin labeling revealed a rounded morphology of AF and NP cells in Col-THA after 1 day of culture, the cells showed an elongated morphology afterwards, suggesting cell adhesion to the polymer network. AF and NP cells proliferated in 2D culture and also when seeded on a Col-THA gel, however there was absence of cell proliferation in the two bioinks, as shown by PicoGreen and EdU assays. Interestingly, we also evidenced a significant contraction of the bioink over time, as a function of the initial cell density (from 0 to 6 million cells/mL), suggesting cell-mediated remodeling of the bioink.

Conclusion. Col-THA bioinks are not cytotoxic for ovine NP and AF cells and promote cell

adhesion. Interestingly, cell seeding induces a bioink contraction upon culture, allowing to control the final dimensions of the bioprinted IVD construct.

keywords: bio-printing, hydrogel, in vitro model, tissue engineering

83767205724

CHARACTERIZATION OF MOLECULAR MECHANISMS REGULATING PLASTICITY OF HUMAN NASAL CHONDROCYTES TO IMPROVE TISSUE REGENERATION

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Introduction: Intervertebral disc (IVD) disease is a common condition characterized by degeneration of one or more discs that separate the vertebrae causing back and neck pain. Current treatments focus entirely on symptom management rather than causal cure. This research focuses on using nasal chondrocytes (NCs) to ameliorate degenerative disc disease (DDD). NCs originate from multipotent cranial neural crest cells (CNCCs) during embryonic development and possess high regenerative capacity. However, NCs would face several challenges such as low oxygen, reduced glucose, increased matrix acidity and elevated levels of proinflammatory cytokines within the DDD microenvironment. The research focuses on identifying genes or markers that enable selection of high performing cell sub-populations within a nasal cartilage biopsy and priming NCs to survive the DDD environment.

Methodology: 200 clones from a fresh nasal biopsy were isolated and differentiation potetential into chondrogenic tissue was assessed. The most and least chondrogenic clones will be subjected to RNA-sequencing to identify a genetic basis for intra-tissue variability. For the priming experiment, NCs were subjected to different “starvation” conditions and then tested for clonogenicity in inflammatory conditions.

Results: When clones from nasal biopsy were subjected to chondro-pellet differentiation, Safranin-O staining showed a wide variety of chondrogenic capacities.This indicates that there exists intra-tissue variability in human nasal cartilage. Chondrogenic and non-chondrogenic clones were isolated based on histological staining intensities, cell distance and morphology. Furthermore, the speed of proliferation of clones was independent of chondrogenicity.
In order to observe the behaviour of good and bad clones under different environmental conditions as compared to standard (4.5g/L glucose in normoxia), NCs were cultured in physiological blood-glucose levels (1g/L glucose) in hypoxia. After 5 days in culture, the rate of proliferation of both chondrogenic and non-chondrogenic clones slowed down in hypoxia in both conditions. Interestingly, while there was little to no difference in cells from the chondrogenic clone in 4.5g/L glucose and 1g/L glucose conditions in hypoxia, the non-chondrogenic clone could not cope in 1g/L glucose in hypoxia. The validity of this phenomenon was confirmed by exposing more non-chondrogenic clones to these experimental conditions. NCs were also ‘primed’ by glucose and lipids (FBS) deprivation. Since the IVD microenvironment is characterised by low oxygen, NCs were cultured in hypoxia. NCs, when deprived of both glucose and lipids, performed the best in normal as well as DDD-mimicking CFU assays as compared to control conditions.

Conclusion: It is clear there exists intra-tissue variability in human nasal cartilage. RNA-sequencing and ATAC sequencing might provide clues to what causes this variability. It is also apparent that non-chondrogenic clones differ in terms of coping with hypoxia and lower levels of glucose. Since the normal environment of nasal chondrocytes is not as harsh and deprived as the IVD, it will be important to find a way to prepare these cells in vitro before injecting them

into the IVD. This research will address the selection of only chondrogenic cells from a nasal biopsy and priming these cells under harsh conditions in vitro to achieve best possible results in vivo.

keywords: Nasal chondrocytes, Degenerative disc disease, Intervertebral disc

31412711406

HYPEROSMOLAR EXPANSION MEDIUM IMPROVES CANINE NUCLEUS PULPOSUS CELL PHENOTYPE

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Lower back pain due to intervertebral disc (IVD) degeneration is a major health problem and often starts in the centre of the IVD, called the nucleus pulposus (NP). NP cells are considered potential candidates for cell-based therapies to induce regeneration of the IVD. However, as the availability of NP cells is limited, extensive in vitro expansion is needed prior to transplantation which results into loss of the NP phenotype hampering therapeutic use of NP cells. A promising strategy to optimize NP cell expansion is to employ physiological stimuli e.g. osmolarity, which is known to decrease during the degenerative process1. Preliminary work on human NP cells indicated that increasing medium osmolarity during expansion has beneficial effects2. Here, we present a comprehensive investigation into the effects of increasing the medium osmolarity during expansion on the phenotype of dog NP cells and their regenerative capacity after re-differentiation in a 3D culture model.
NP cells of 6 Beagle donors (mildly degenerated IVDs) were expanded for 2 passages in expansion medium with a standard osmolarity of 300 mOsm/L or adjusted to 400 or 500 mOsm/L (mimicking levels in the healthy IVD). Following expansion, cells from each condition were re-differentiated for 14 days in 3D microaggregates in chondrogenic medium with a standard osmolarity of 300 mOsm/L to mimic placing them back in the degenerated in vivo IVD environment. Readout parameters during expansion were focussed on cell morphology, phenotype and proliferation rate. After re-differentiation we assessed the phenotype and regenerative capacity of the NP cells.
NP cells expanded at an increased expansion medium osmolarity (500 mOsm/L) were able to maintain an in vivo-like, rounded (less fibroblast-like) cell morphology at the expense of a lower cell proliferation rate. Further, expansion in 500 mOsm/L lead to a significant increase of several healthy NP cell and progenitor markers at gene expression level (KRT18, ACAN, COL2, CD73, CD90) and protein level (ACAN, PAX1, CD24, TIE2, CD73) compared to NP cells expanded in standard medium osmolarity (300 mOsm/L). Immunohistochemical stainings for ACAN, PAX1, CD24 and TIE2 and qualitative and quantitative assessment of extracellular matrix deposition showed that the NP cells expanded at 500 mOsm/L were able to retain their NP cell phenotypic markers and regenerative capacity in the 3D re-differentiation culture model.
Our findings show that increasing medium osmolarity improves the NP cell phenotype during in vitro expansion, and with that, facilitates the potential of NP cells to be used for regenerative cell-based treatments.

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keywords: intervertebral disc, regeneration, cell-based therapies

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SELF-ASSEMBLING PEPTIDE HYDROGELS FOR NUCLEUS AUGMENTATION OF THE INTERVERTEBRAL DISC

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INTRODUCTION
Lower back pain affects 80 % of adults and has estimated costs up to 1 – 2 % of GDP1,2. Current surgical treatments include spinal fusion, but outcomes are poor with reoperation required in 20% of patients3. An emerging and more regenerative approach is nucleus augmentation, which aims to restore healthy disc anatomy and biomechanical function through the delivery of a biomaterial into the nucleus of a degenerated intervertebral disc. Self-assembling peptides (PEP) can be used with glycosaminoglycans (GAG) to form a PEP-GAG hydrogel and tuned to improve the biocompatibility and mechanical properties for use as a nucleus augmentation material. This work focuses on assessing three different peptides and their viability for nucleus augmentation.

EXPERIMENTAL METHODS
All samples contain peptide (20 mg/ml) with or without chondroitin sulfate (136 mg/ml).
Contact, extract, and filter diffusion assays were used to assess PEP-GAG hydrogel and counterion cytotoxicity with L929 and BHK cells.
Small amplitude oscillatory shear rheology was used to assess mechanical properties after different modes of delivery.
Peptide samples were made at a range of concentrations in D2O to determine the critical concentration (c*) for self-assembly using 1H NMR.
CryoSEM and FIB-SEM images were obtained using FEI – Helios G4 CX Dual beam FIBSEM.

RESULTS AND DISCUSSION
Cytotoxicity testing of different PEP-GAG hydrogels showed some slight cytotoxicity in the indirect and extract assays but not in the direct contact assay with the three different peptides behaving similarly across the assays. A greater difference in sample cytotoxicity was seen when comparing trifluoroacetate (TFA), acetate and HCl counterions with HCl showing the least cytotoxicity compared to TFA and acetate.
As the end polar amino acids change from ser-ser to glu-ser to glu-glu the c* decreases as a result of the increased number and strength of intermolecular hydrogen bonding. When injected down different needle lengths, gauges and designs, there was a small reduction in gel stiffness (G’) when the needle design was changed, however this small change was not clinically relevant. CryoSEM images showed that all three PEP-GAG hydrogels were able to form fibrous networks.

CONCLUSION
All three peptides investigated were able to form PEP-GAG hydrogels with low levels of cytotoxicity. Changing the end polar amino acid between glutamine and serine allows for tuning of the material properties. The mechanical properties as determined by rheology suggest that the PEP-GAG hydrogels are suitable for nucleus augmentation. The use of a minimally invasive clinical delivery device slightly reduces the gel stiffness but provides the advantage of reduced damage to the annulus fibrosus and simultaneous injection of the two components to allow better mixing in situ. The PEP-GAG hydrogels offer a promising new approach to repair the degenerated intervertebral disc to treat lower back pain.

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keywords: Hydrogel, nucleus augmentation, biomaterial, self-assembling peptides

PS68
Human brain organoids versus assembloids approach for neurodevelopmental studies

83767247455

IN VITRO 3D MODELING OF THE HUMAN DOPAMINERGIC SYSTEM

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The dopaminergic system is vital for the control of both motor control (nigrostriatal pathway, A9 dopaminergic neurons), and reward (mesocorticolimbic pathway, A10 dopaminergic neurons). Both pathways are associated with different types of diseases: the nigrostriatal pathway is most commonly associated with Parkinson's disease, whereas the mesocorticolimbic pathway becomes dysregulated in addiction. During development, dopaminergic neurons emerge from the floor plate of the ventral midbrain, from where they project to the striatum (A9 and A10) and cortex (A10).
Brain organoids are a powerful iPSC or hES derived system to model both human-specific neurodevelopmental as well as neuropathological aspects which to date could not reliably be studied due to the lack of appropriate model systems.
To model the above mentioned disorders on the circuit level, we have developed an assembloid culture model recapitulating the dopaminergic system. We confirm the faithful recapitulation of development and differentiation of the appropriate cell types by both antibody staining and single cell RNA sequencing. Using a recently published 3D IHC and tissue clearing protocol, we could demonstrate that dopaminergic neurons innervate the striatal and cortical part of the assembloids and mature structurally over time. Currently, we perform experiments to investigate maturation from structural to functional synapses of these dopaminergic long range projections, with the aim to study dysregulation of the dopaminergic system and its effects in striatal and cortical neurons.
Using our assembloid culture model of the dopaminergic system will provide further insights into both the development of the dopaminergic system, but also in diseases associated with dopaminergic neurons, such as addiction and Parkinson's disease.

keywords: dopamine, neurodevelopment, organoid

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LINKING ABNORMAL CA2+ SIGNALING AND THE UNFOLDED PROTEIN RESPONSE WITH HUNTINGTON'S DISEASE PATHOLOGY IN BOTH IPSC-DERIVED MSNS NEURONS AND STRIATAL ORGANOIDS FROM HD PATIENTS

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Introduction
Huntington's disease (HD) is a progressive neurodegenerative disorder with autosomal-dominant heritability characterized by the aggregation of mutant huntingtin (mHTT) protein. HD is described by the region-specific neuronal degeneration of medium spiny neurons (MSNs) in the striatum, elevated Ca2+ signaling, and dendritic spine pathology. However, the pathway connecting disturbed Ca2+ signaling, which is suggested as an early event in HD pathology, and the neuronal death is unclear. We found that huntingtin-associated protein 1 isoform A (HAP1A), a binding partner of mHTT, dysregulates Ca2+ dynamics in the endoplasmic reticulum (ER) through increased activation of inositol-(1,4,5)triphosphate receptor 1 (IP3R1). Opening of IP3R1 via HAP1A elevates store-operated calcium entry (SOCE) in YAC128 MSN cultures, HD mouse model for in vitro studies (Czeredys et al. 2018). Enhanced SOCE activity leads to striatal synaptic loss. ER stress is also dysregulated in HD models. Therefore, we hypothesized that dysregulated (via HAP1A) Ca2+ signaling is responsible for the activation of unfolded protein response (UPR), which in turn leads to neurodegeneration. To test our hypothesis, we will use both iPSCs-based GABAergic MSNs and striatal organoids derived from HD patients' fibroblasts from different HD onsets.

Methodology
Fibroblasts lines with different stages of Huntington's disease progression including presymptomatic HD, early manifest HD, manifest HD onset, as well as juvenile HD and appropriate controls were cultured and SOCE was measured using single-cell Ca2+ measurements. Fibroblast lines were reprogramed to iPSCs lines using STEMCCA Lentivirus Reprogramming Kit. Established iPSCs lines were characterized using different methods including karyotyping, STR analysis, sequencing, RT-PCR, immunofluorescence, and embryonic bodies formation assay. To test the project hypothesis iPSCs lines are being differentiated into iPSCs-based GABAergic MSNs and striatal organoids (Latoszek and Czeredys, 2021).

Results
Changes in SOCE in fibroblasts from different HD onsets were observed. We found that our iPSCs lines showed alkaline phosphatase activity and normal colony morphology as well as they are mycoplasma free. We detected that the reprogramming process does not affect the karyotype of iPSCs lines. Sequence consistency of CAG repeats in the HTT gene was demonstrated both by sequencing and PCR between fibroblasts and iPSC lines. The concordance of selected STRs between iPSCs and fibroblast lines has been shown. RT-qPCR analysis and immunofluorescence revealed the expression of pluripotency markers in iPSC lines. We also obtained embryonic bodies from iPSCs lines which were characterized by the expression for markers of three germ layers by RT-PCR and immunofluorescence.

Conclusion
iPSCs-based GABAergic MSNs and striatal organoids obtained from HD patients' fibroblasts from different HD onsets will be an important tool to study the role of abnormal Ca2+ signaling and UPR in the neurodegeneration of HD. The present work is a significant step towards this goal.
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keywords: Huntington's disease, SOCE, calcium signaling, iPSCs, organoids

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THE EFFECT OF MITOCHONDRIAL BIOGENESIS INDUCTION THROUGH A7NACHR AGONIST ON THE CELL FATE AT THE EARLY DEVELOPMENTAL STAGE OF HUMAN CORTICAL ORGANOID

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Introduction: The brain organoids derived from human-induced Pluripotent Stem Cells (iPSC) provide a tool to model and study human brain development and its pathology. Up to date reports regarding the role of mitochondrial biogenesis (MB) in cell-type specification during in vitro human cortical development are not available. By inducing mitochondrial biogenesis at different developmental stages of cortical organoid - Embryonic Body (EB), Neurosphere (NS), and Cortical Organoid (CO), we aim to elucidate its role in neurodevelopment.
The main objective of this study was to determine the effect of MB induction on cell fate commitment specifically in the Embryonic Body structures, resembling the earliest developmental stage of human cortical development.

Methods: In this study brain region-specific cortical organoids were derived from human iPSCs with EBs as the earliest stage of development. EBs were maintained in Essential 6 media for 13 days, to induce spontaneous differentiation and expression of 3-germ layers markers. MB was induced by alpha-7 nicotinic acetylcholine receptor (α7nAChR) agonist, PNU-282987 for seven days. The toxicity of different doses of PNU-282987 (5, 10, 20, 50, 100 µM) was evaluated by AlamarBlue and CellTiter-3D Glo viability assays and an apoptotic assay using CellEvent™ caspase-3/7 green detection reagent and Hoechst. The level of mitochondria biogenesis was determined by the ratio of mtDNA copy number to genomic DNA, ND1/HBB and ND5/SERPINA1 via qPCR. The presence of 3-germ layers markers – FOXA2 for endoderm, α-SMA for mesoderm as well as Pax6 and Nestin for ectoderm were evaluated using immunofluorescence labeling and RT-PCR analysis for the expression of markers at cellular and genomic level respectively. Proliferation was evaluated by calculating the number of Ki67-positive cells after 7-day induction of MB.

Results: The cell viability assays using resazurin and luciferin indicating live cells as well as apoptotic assay using Cas3/7 detection reagent with Hoechst, indicating the percentage of apoptotic cells, revealed that all tested concentrations of PNU-282987 had no toxic effect on the EBs formation. Furthermore, the measurement of the ratio of mtDNA to genomic DNA revealed that while most of the used concentrations resulted in increased mtDNA copy number, the concentration of 10 µM induced MB more effectively in statistically significant manner. The early, Embryonic Body stage of cortical organoid development treated with 10 µM PNU-282987 showed significant increase in cell proliferation, as revealed by the calculation of Ki67 positive cells. Lastly, changes in expression of ectodermal markers- Pax6 and Nestin as compared to endodermal and mesodermal markers at genomic level, revealed that in 3D Embryonic Bodies, MB induction drives cell fate commitment into ectoderm layer. Quantitative evaluation of the

fluorescence intensity of the ectodermal markers at cellular level supports the notion of the influence of MB induction in cell fate specification during the early stage of human cortical organoid.
Conclusions: Indu
ction of Mitochondrial Biogenesis in EB stage of cortical organoid development guides cell fate specification into neuroectodermal lineage. Mimicking human early neurodevelopment with 3D cortical organoid model provides a useful framework to study the role of mitochondrial biogenesis in developmental stage-dependent cell fate specification.

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keywords: embryonic bodies, cortical organoids, hiPSCs, mitochondrial biogenesis, cell fate

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