Regenerative Medicine

2015 Vol. 10 No. 07s

ISSN 1746-0751

• Meeting Abstracts
World Conference on Regenerative Medicine 2015
Congress Center Leipzig, Leipzig, Germany
October 21–23 2015

Future Medicine part of fsg

Regenerative Medicine

2015 *Regenerative Medicine* 10(7s)

Regenerative Medicine

Regenerative Medicine

†ECS is also an investigator on the BRITS project funded by the Technology Strategy Board under their Regenerative Medicine program: Value Systems and Business Modelling.

Indexing:
MEDLINE/Index Medicus, Science Citation Index Expanded, Biotechnology Citation Index®, Journal Citation Reports, Biological Abstracts, BIOSIS Previews, EMBASE/Excerpta Medica, Chemical Abstracts

Impact factor: 3.5 (2013)

Advertising Enquiries
Dionne Murray, Business Development Manager
d.murray@futuremedicine.com
Editorial Enquiries
Laura Dormer, Senior Manager: Commissioning
l.dormer@futuremedicine.com
Subscription Enquiries
Dominik March, Subscription Sales Manager
d.march@futuremedicine.com
Reprint Enquiries
Sam Cavana, Reprint Sales Manager
s.cavana@futuremedicine.com
Permissions Enquiries
Adina-Stefana Mois, Publishing Administrator
a.mois@futuremedicine.com

www.futuremedicine.com

Future Medicine part of fsg

Aims & Scope

"…a forum to address key advances and challenges in stem cells research and regenerative medicine…"

Improved healthcare has resulted in dramatic demographic changes in developed countries, causing an increase in the prevalence of diseases associated with aging. Many significant human diseases arising from the loss or dysfunction of specific cell types in the body, such as Parkinson's disease, diabetes and cancer, are becoming increasingly common.

Stem cell research and regenerative medicine offers unique opportunities for developing new therapeutic approaches to prevent and treat these debilitating and life-threatening diseases, and new ways to explore fundamental questions of biology. Gradually, the curative and regenerative potential that lies in harnessing stem cells and other regenerative strategies is being realized.

Regenerative Medicine (ISSN: 1746-0751) provides a forum to address the important challenges and advances in stem cell research and regenerative medicine, delivering this essential information in concise, clear and attractive article formats – vital to an increasingly time-constrained community.

Despite substantial developments in our knowledge and understanding of regenerative biology and stem cells, the field is in its infancy. The next few decades will unveil the true potential of this emerging specialty. *Regenerative Medicine* will provide a critical overview of these advances as they unfold and explore their potential relevance in the clinical setting.

The journal covers emerging strategies to replace or regenerate human cells, tissues or organs to restore or establish normal function, including:

- *Bench-to-bedside translation and scale-up of stem cell and regenerative medicine therapies*
- *Potential applications for stem cell-based strategies in pathological conditions*
- *Stem cell pluripotency and emerging technologies*
- *Tissue engineering and artificial organ development*
- *Medical device and artificial organ development*
- *Regulatory and reimbursement issues*
- *Ethical and legal perspectives*

Subscription options

Institutional subscriptions
Regenerative Medicine is available in print, electronic or print and electronic formats, and pricing will depend on your organization type (academic, corporate, hospital, etc). Please contact **info@futuremedicine.com** for more details.

Global e-access licenses are available on request and attract considerable discounts from standard site license fees. For further details on global access licenses, please contact **sales@futuremedicine.com**

Consortia pricing
Regenerative Medicine welcomes discussion with all consortia, and offers flexible packages and discounted prices. If you have specific questions or would like a quote please contact **info@futuremedicine.com** for more details.

Personal subscriptions
Personal subscriptions are currently available to all Future Medicine journals. Payment must be made from a personal credit card registered to a home address. Print subscriptions will only be sent to a personal address. Please contact **info@futuremedicine.com** for our personal order form, or order online at **www.future-science-group.com/subscriptions**.

Ordering information

Please contact your local sales representative to place an order:

Worldwide
Future Medicine Ltd
Unitec House, 2 Albert Place,
London, N3 1QB, UK
T: +44 (0)20 8371 6090
F: +44 (0)20 8343 2313
E: subscriptions@futuremedicine.com

North America
E: sales.us@future-science.com

Middle East
Naseej
T: +966 1 477 0477; ext. 232
E: a.alkredees@naseej.com

Latin America & the Caribbean
dotLib
T: +55 (21) 3431 3430
E: info@dotlib.com

Asia (excluding Korea, China & Japan)
Roslinda M. Razi
T: +65 3153 0633
E: r.razi@futuremedicine.com

Korea
Shinwon Datanet Inc.
T: +822 326 3535
E: info@shinwon.co.kr

China
Charlesworth China
T: +86 (0)106 779 1601
E: sales@charlesworth.com.cn

Japan
USACO Corporation
T: +81 33 505 3257
E: marketing@usaco.co.jp

Subscription rates (2015)

	Print & Online			Online only		
Journal (8 issues)	£ GBP	€ EUR	$ US	£ GBP	€ EUR	$ US
Academic/Hospital	1005	1340	1690	895	1185	1495
Corporate/Government	Please contact **info@futuremedicine.com** for more details					

Reprints

Article reprints are available through our reprint service. Please contact: **reprints@futuremedicine.com**

Future Medicine titles endorse the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, issued by the International Committee for Medical Journal Editors, and Code of Conduct for Editors of Biomedical Journals, produced by the Committee on Publication Ethics.
This information is also available at www.futuremedicine.com

Manuscript submission & processing

Future Medicine titles publish a range of article types, including solicited and unsolicited reviews, perspectives and original research articles. Receipt of all manuscripts will be acknowledged within 1 week and authors will be notified as to whether the article is to progress to external review. Initial screening of articles by internal editorial staff will assess the topicality and importance of the subject, the clarity of presentation, and relevance to the audience of the journal in question. If you are interested in submitting an article, or have any queries regarding article submission, please contact the Managing Commissioning Editor for the journal (contact information can be found on our website at: www.futuremedicine.com. For new article proposals, the Managing Commissioning Editor will require a brief article outline and working title in the first instance. We also have an active commissioning program whereby the Commissioning Editor, under the advice of the Editorial Advisory Panel, solicits articles directly for publication.

External peer review: Through a rigorous peer review process, Future Medicine titles aim to ensure that reviews are unbiased, scientifically accurate and clinically relevant. All articles are peer reviewed by three or more members of the International Advisory Board or other specialists selected on the basis of experience and expertise. Review is performed on a double-blind basis – the identities of peer reviewers and authors are kept confidential. Peer reviewers must disclose potential conflicts of interests that may affect their ability to provide an unbiased appraisal (see Conflict of Interest Policy below). Peer reviewers complete a referee report form, to provide general comments to the editor and both general and specific comments to the author(s).

Where an author believes that an editor has made an error in declining a paper, they may submit an appeal. The appeal letter should clearly state the reasons why the author(s) considers the decision to be incorrect and provide detailed, specific responses to any comments relating to the rejection of the review. Further advice from members of the journal's Editorial Advisory Panel external experts will be sought regarding eligibility for re-review.

Revision: Most manuscripts require some degree of revision prior to acceptance. Authors should provide two copies of the revised manuscript – one of which should be highlighted to show where changes have been made. Detailed responses to reviewers' comments, in a covering letter/email, are also required. Review manuscripts may be accepted at this point or may be subject to further peer review. The final decision on acceptability for publication lies with the journal editor.

Post-acceptance

Accepted review manuscripts are edited by the in-house Future Medicine editorial team. Authors will receive proofs of their article for approval and sign off and will be asked to sign a transfer of copyright agreement, except in circumstances where the author is ineligible to do so (e.g. government employees in some countries).

Author disclosure & conflict of interest policy

Authors must state explicitly whether potential conflicts do or do not exist (e.g. personal or financial relationships that could influence their actions) and any such potential conflict of interest (including sources of funding) should be summarized in a separate section of the published review. Authors must disclose whether they have received writing assistance and identify the sources of funding for such assistance. Authors declaring no conflict of interest are required to publish a statement to that effect within the article.

Authors must certify that all affiliations with or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in their manuscript have been disclosed. Please note that examples of financial involvement include: employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending and royalties. This is list is not exclusive of other forms of financial involvement. Details of relevant conflicts of interests (or the lack of) must be declared in the 'Disclosure' section of the manuscript for all listed authors.

External peer reviewers must disclose any conflicts of interest that could bias their opinions of the manuscript, and they should disqualify themselves from reviewing specific manuscripts if they believe it appropriate. Should any such conflict of interest be declared, the journal editor will judge whether the reviewer's comments should be recognized or will interpret the reviewer's comments in the context of any such declaration.

Authorship & contributorship

All authors should meet the ICMJE authorship criteria as follows: (1) they have provided significant input into the design and concept of the study that is the subject of the paper or were pivotal in the acquisition, analysis or interpretation of data; (2) they drafted the paper or were involved in making significant revisions; and (3) they approved the final version of the paper. The corresponding author should accept direct responsibility for the manuscript, including liaising with all authors for their feedback and statements of disclosure, and will be responsible for approval of the final version prior to publication.

Ethical conduct of research

For studies involving data relating to human or animal experimental investigations, appropriate institutional review board approval is required and should be described within the article. For those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki should be followed. For investigations involving human subjects, authors should explain how informed consent was obtained from the participants involved.

Patients' rights to privacy

Patients have a right to privacy that should not be infringed without informed consent. Identifying information should not be included unless the information is essential for scientific purposes and the patient (or parent or legal guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. When informed consent has been obtained it should be indicated in the manuscript.

In attempting to maintain patient anonymity, identifying details should be omitted where they are not essential. However, patient data should never be amended or falsified. Informed consent should be obtained whenever there is any doubt that anonymity can be assured.

Use of personal communications & unpublished data

Where an individual is identified within a review as a source of information in a personal communication or as a source for unpublished data, authors should include a signed statement of permission from the individual(s) concerned and specify the date of communication.

Clinical trial registration

Future Medicine titles prefer to publish clinical trials that have been included in a clinical trials registry that is accessible to the public at no charge, is electronically searchable, is open to prospective registrants and is managed by a not-for-profit organization, such as www.clinicaltrials.gov (sponsored by the United States National Library of Medicine). Whilst referees will take registration status into account, all well designed and presented trials and corresponding data will be considered for publication.

Errata/corrigenda

Mistakes by either editor or author should be identified wherever possible and an erratum or corrigendum published at the earliest opportunity. We will attempt to contact the author of the original article to confirm any error, and publish an appropriate erratum or corrigendum at the earliest opportunity.

Permissions for reproduced or adapted material

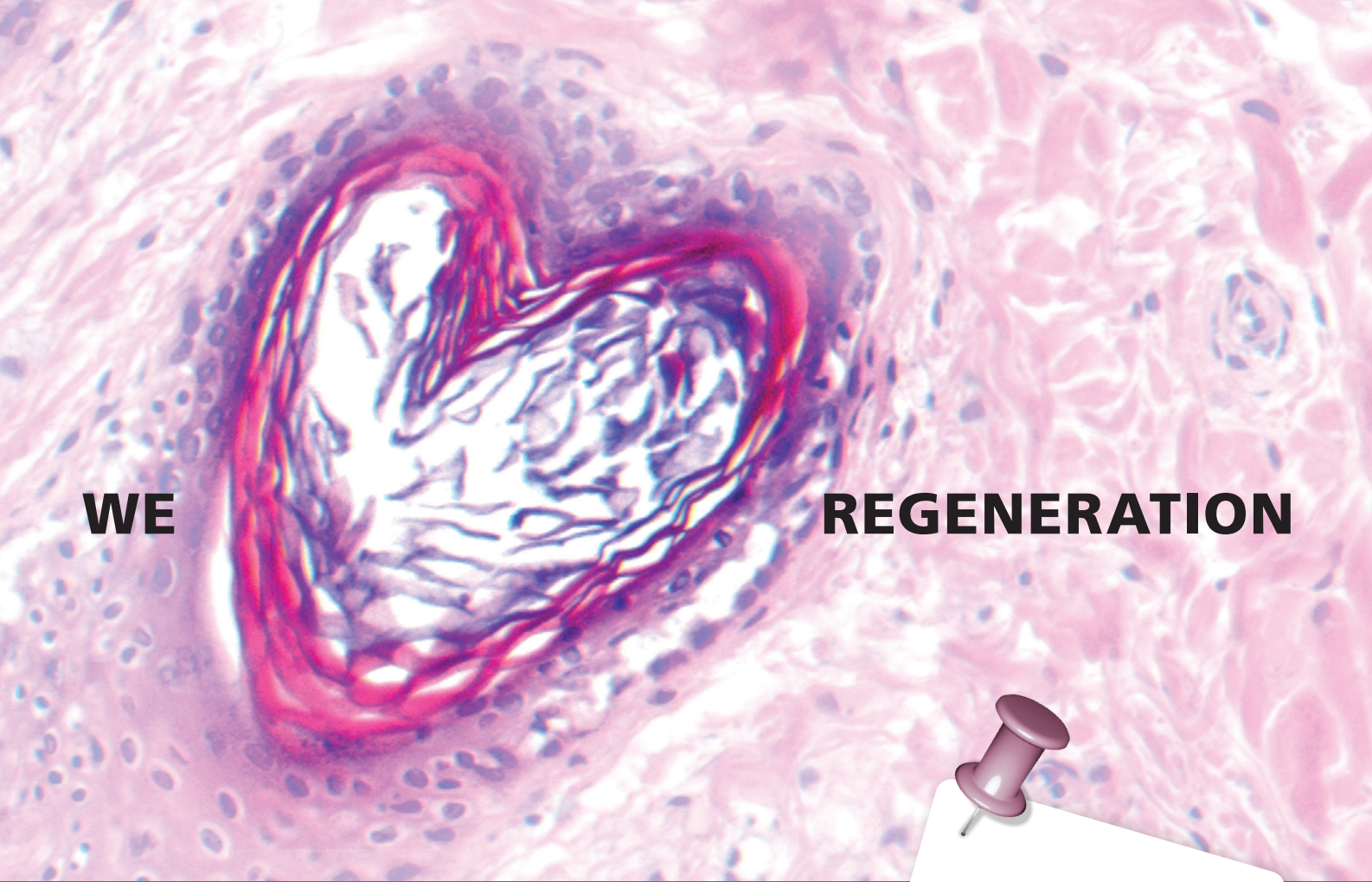
Authors must acknowledge the origin of all text, figures, tables or other information that has been adapted or reproduced from other publications. Authors must provide a copy of the original source documents and should submit permission from the authors of the original work and the original publishers for unlimited use in all markets and media (that includes both electronic and print use in any language).

Duplicate publication/submission & plagiarism

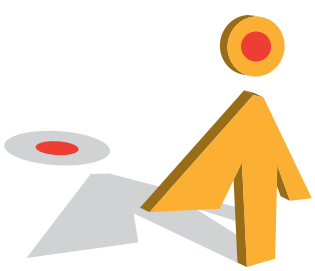
All manuscripts submitted to Future Medicine titles are considered for publication on the understanding that they have not been published previously elsewhere or are under consideration for publication elsewhere. The journal may, however, consider republication of a paper previously published in a language other than English, subject to prominent disclosure of the original source and with any necessary permission. Authors will be asked to certify that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under their authorship has been published or is being considered for publication elsewhere, except as described in an attachment, and copies of closely related manuscripts are provided. All submitted articles will be evaluated using plagiarism detection software, which compares the submitted manuscript with full text articles from all major journals databases and the internet. The use of published or unpublished ideas, words or other intellectual property derived from other sources without attribution or permission, and representation of such as those of the author(s) is regarded as scientific misconduct and will be addressed as such.

Misconduct

If misconduct by authors or reviewers is suspected, either pre- or post-publication, action will be taken. An explanation will be sought from the party or parties considered to be involved. If the response is unsatisfactory, then an appropriate authority will be asked to investigate fully. Future Medicine will make all reasonable attempts to obtain a resolution in any such eventuality and correct the record or archive as necessary.



SAVE THE DATE



world conference on **regenerative medicine**

[Germany | Leipzig | Autumn 2017]

WWW.WCRM-LEIPZIG.COM

Follow us on Twitter! @FraunhoferIZI

www.facebook.com/
WorldConferenceOnRegenerativeMedicine

9th International Symposium on NEUROPROTECTION NEUROREPAIR | 2016

April 19 – 22, 2016 | Leipzig, Germany

Unique meeting location: LEIPZIG ZOO

Photo: Zoo Leipzig

SELECTED TOPICS:

- Translational stories: from bench to beside – and back
- Improving stroke care: imaging and novel diagnostics
- A point of view: current controversies in research and clinics
- Post-stroke immunity: Has the time come for clinical trials?
- New connections: neurorehabilitation and human brain plasticity
- Along the supply chain: the neurovasculature in stroke and dementia

… and many more!

FEATURING NEW FORMATS:

Minisymposia
Plenary discussion
The Science Slam

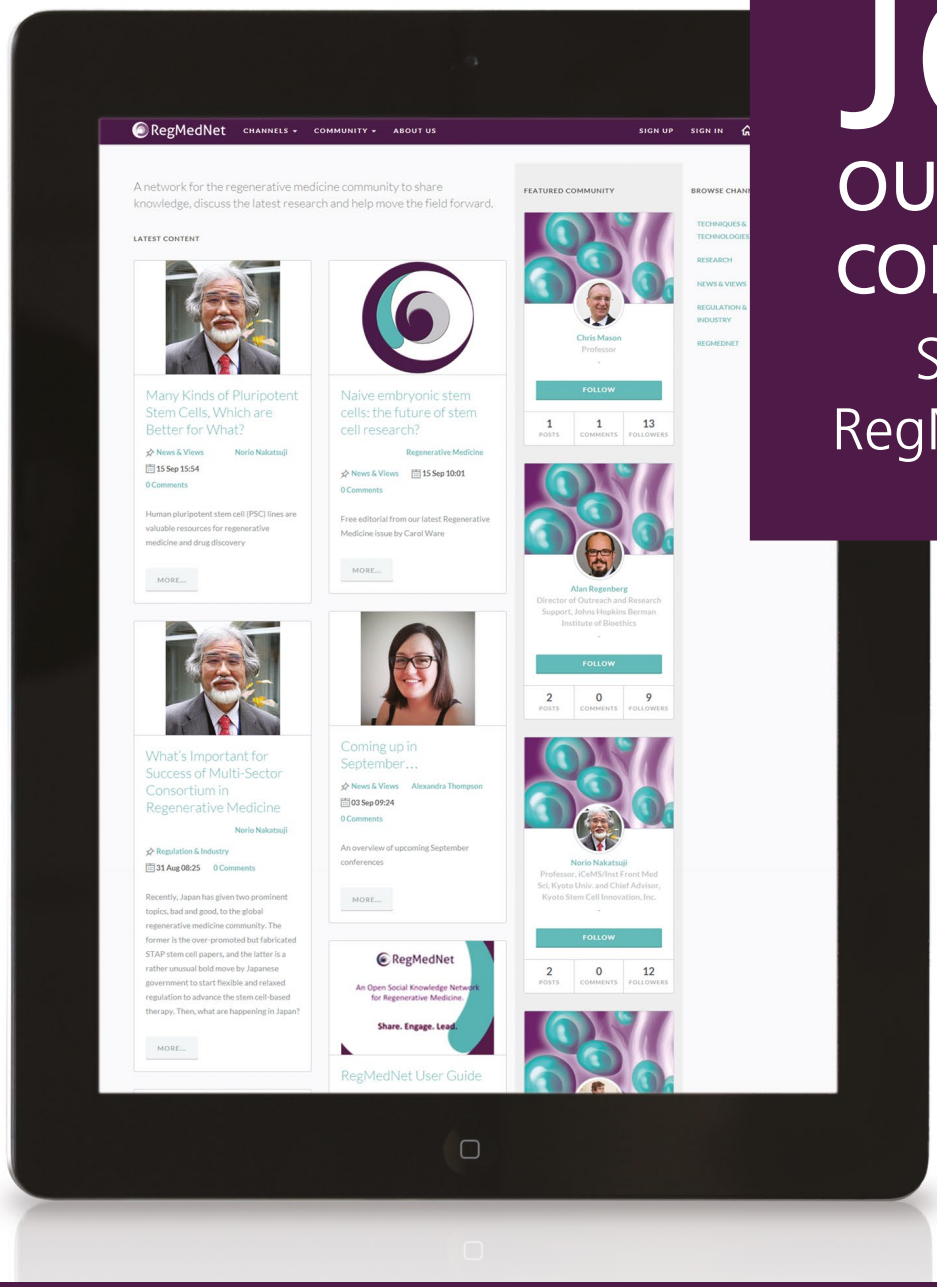
CONFERENCE COORDINATOR
Johannes Boltze, MD, PhD
Fraunhofer Institute for
Cell Therapy and Immunology

THE DEADLINE FOR ABSTRACT SUBMISSION IS NOVEMBER 30, 2015.

neurorepair-2016.de

JOIN
OUR ONLINE COMMUNITY

Sign up at RegMedNet.com



RegMedNet: have you joined?

RegMedNet is a unique and unparalleled platform for the regenerative medicine community to share insights, discuss the latest research, and help move the field forward. Join our newest expert network today.

Powered by RegMedNet

fsg

OP-002 Human organotypic liver microtissue formation in a hollow-fiber membrane bioreactor

*H. M. M. Ahmed[1,2], S. Salerno[1], L. Giorno[1], L. De Bartolo[1]

[1]Institue on Membrane Technology, Rende, Italy
[2]University of Calabria, Department of Environmental and Chemical Engineering, Rende, Italy

Objectives

Loss of liver functions due to disease, injury or accidents is a major life-threatening condition affecting millions worldwide. Thus far the only available treatment is organ transplantation; however, due to the scarcity of organ donors alternative approaches have been sought. Various non- biological and biological approaches have been adapted but with limited success, due to the failure of the earlier to replace the liver's synthetic and metabolic functions, and the difficulty of implementing the latter in clinical setting. Recently, Bioartificial liver devices (BAL) have been investigated as a promising alternative. One limitation that needs to be overcome for a BAL to be efficient is the short lifespan of hepatocytes cultured *in vitro*.

Materials and Methods

To this end, in the present study a co-culture of human hepatocytes together with non-parenchymal cells, namely stellate and sinusoidal endothelial cells, was realized utilizing a hollow fiber membrane bioreactor. Sinusoidal endothelial, stellate cells and hepatocytes were seeded in a subsequent order on polyethersulfone (PES) as well as modified polyetheretherketone (PEEK-WC) hollow fiber membranes. As a positive control, human hepatocytes were seeded alone on PEEK-WC hollow-fiber membranes. Liver-specific metabolic and synthetic functions were assessed in terms of diazepam metabolism, urea synthesis and albumin production.

Results

Functional analysis showed that hepatocytes in co-culture maintained their liver-specific functions throughout the experiment, with higher levels as compared to the hepatocyte monoculture. Scanning electron microscope images clearly demonstrated the formation of tissue-like structures on the surface of the hollow-fiber membranes. Confocal laser scanning microscope images confirmed that these are indeed microtissues made up of a mixture of all 3 cell types with hepatocytes constituting the majority of those cells.

Conclusion

In this study, a 3D organotypic co-culture of human hepatocytes and non-parenchymal cells was realized in a hollow-fiber membrane bioreactor providing a valuable tool for prolonging hepatocyte functionality. This co-culture model that mimics, to an extent, the *in vivo* microenvironment of the human liver offering a promising tool for *in vitro* toxicity studies of new pharmaceuticals and possibly the development of a functional BAL in the near future.

Disclosure

This work is sponsored by Marie Curie ITN - BIOART Project.

OP-003 Evaluation of jaw periosteal cell mineralization by raman micro-spectroscopy

*D. Alexander[1], E. Brauchle[2], D. Carvajal Berrio[2], M. Rieger[1], K. Schenke-Layland[2,3], S. Reinert[1]

[1]University Hospital Tübingen, Dept. of Oral and Maxillofacial Surgery, Tübingen, Germany
[2]Fraunhofer Institute for Interfacial Engineering and Biotechnology, Dept. of Cell and Tissue Engineering, Stuttgart, Germany
[3]University Hospital Tübingen, Dept. of Women's Health, Tübingen, Germany

Objective

We previously identified the mesenchymal stem cell antigen-1 (MSCA-1) to hallmark osteoprogenitor cells derived from the jaw periosteum. Under serum-free culture conditions, we detected high proliferation rates of this subpopulation. The aim of the present study was to investigate whether the marker-free technology of Raman spectroscopy is suitable to evaluate the osteogenic potential of jaw periosteal cells. Therefore, MSCA-1+ cells were analysed under serum-containing (DMEM) and -free (MC) culture conditions.

Material and methods

Within each analysed cell culture dish, Raman spectra from 100 random points were measured. Principle component analysis (PCA) of Raman spectra was performed and cell mineralization in either one or two spectral components (PCs) for two out of three donors was detected. PCA was carried out to identify the spectra containing Raman signals from bone material. The quality of formed mineral crystals was assessed by calculating following ratios: mineral-to-matrix, carbonate-to-phosphate, collagen maturity and hydroxyapatite crystallinity.

Results

We detected by both approaches, Raman micro-spectroscopy and fluorescent OsteoImage or Alizarin stainings periosteal cell mineralization in dishes with glass bottom. However, the degree of cell mineralization was not always consistent with calcification detected within cell culture plates of other format. Data evaluation of recorded Raman spectra revealed higher crystallinity, higher collagen maturity and higher mineral-to-matrix ratios in cell monolayers cultured under FCS-containing compared to FCS-free conditions.

Conclusion

Raman micro-spectroscopy provides a suitable tool for studying the extent of MSCA-1+ periosteal cell mineralization. However, analyses of mechanical properties of formed crystals will be of further interest to be able to make a reliable statement regarding *in vitro* cell mineralization.

OP-005

Longitudinal assessment of focal de- and remyelination of human myelin in a human chimeric mouse model by quantitative MRI

*A. Arnold[1,2], Q. Li[1,2], J. Zhang[1,2], W. Li[1,3], G. Liu[1,3], M. Janowski[1,2,4,5], P. Walczak[1,2]

[1]Johns Hopkins University School of Medicine, Dept. of Radiology and Radiological Science, Baltimore, United States
[2]Institute for Cell Engineering, Johns Hopkins University, Cellular Imaging Section, Baltimore, United States
[3]Kennedy Krieger Institute, F.M. Kirby Research Center for Functional Brain Imaging, Baltimore, United States
[4]Polish Academy of Sciences, NeuroRepair Department, Mossakowski Medical Research Centre, Warsaw, United States
[5]Polish Academy of Sciences, Department of Neurosurgery, Mossakowski Medical Research Centre, Warsaw, United States

Objective

A wide range of neurological disorders result in loss or dysfunction of myelin. Animal models are used to develop treatment, but they only partially resemble the nature of human diseases. In an effort to develop a humanized mouse model that facilitates interrogating function of human myelin *in vivo,* we transplanted human glial-restricted progenitor cells (hGRPs) into neonatal immune-/myelindeficient shiverer mice. Over time, the endogenous mouse brain macroglial populations become substantially replaced by engrafted hGRPs and after one year, mouse brains are practically full chimeras with astrocytes and myelinating oligodendrocytes of human origin. The goal of our study was to evaluate with MRI the dynamics of focal de-/remyelination of human myelin in chimeric (hGC) in comparison to wild type.

Material and Methods

Human fetal GRPs were transplanted bilaterally into both ventricles of neonatal shiverer mice (P1-3). The myelination process of transplanted mice was followed with serial MR imaging. MRI of grafted mice and control groups was performed for up to 680 days using Bruker horizontal 11.7T scanner. 680-days-old human GC and wild type mice were placed into a stereotactic injection frame. A hole was drilled in the right skull to inject 1 µl of 1% L-α-lysophosphatidylcholine (LPC) solution into the corpus callosum using a 33-G Hamilton needle. De-/remyelination were monitored with MRI (MTR, DTI, and T2) for over one month (seven time points). In post-mortem analysis performed at three time points, immunohistochemistry (IHC) confirmed de-/remyelination with myelin basic protein (MBP) and eriochrome staining, repopulation with glial progenitor cells with Olig2 and activated microglia infiltration with Iba1.

Results

After focal LPC-induced demyelination into the right ventricle of our human GC and wild type mice, we monitored the repopulation and remyelination efficiency of GRPs in areas of focal demyelination with longitudinal MRI. The focal lesion generated from LPC injection affects only a small, limited area in the corpus callosum which was confirmed by T2 weighted images in the LPC-injection area compared to the contralateral side in human GC and wild type mice. After 3 DPL T2-weighted imaging showed clear evidence of inflammation in wild type and human GC mice in the ipsilateral side which was gone after 8 DPL. The remyelination process could be longitudinal monitored with T2 weighted imaging after 17 DPL. The tendency for remyelination properties over time had the same efficiency in both treated mice groups. Indeed, no fully remyelination could be monitored after 40 DPL neither for wild type or human GC mice. No clear difference for de-/remyelination was monitored with DTI or MTR. After sacrificing the analyzed mice at 40 DPL, we could confirm the remyelination with staining against MBP. Further, we observed with erichorome staining a reduction in the corpus callosum size in the ipsilateral hemisphere. The size reduction indicates axonal damage after LPC injection which was confirmed with IHC staining for SMI-32 and SMI-31 at 3 and 8 DPL. However, high populations of Iba1 and Olig2 positive cells were accumulated in the lesion area on both earlier time points.

Conclusion

We have shown that humanized chimeric mice are suitable for studying de-/remyelination. Similar rate of recovery in chimeras and control mice is surprising taken significantly slower myelination human cells in development. Evidence for axonal loss is a limitation of the LPC model.

OP-008 Xeno-free-expanded human mesenchimal stromal cells preserve their chemotactic and immunomodulatory properties

*A. Blazquez-Prunera[1,2,3,4], C. R. Almeida[1,2], M. Barbosa[1,2,4]

[1]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal
[3]Faculdade de Engenharia, Universidade do Porto, Porto, Portugal
[4]ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Objective

Culture of human Mesenchymal stromal cells (hMSC) in xeno-free conditions is a necessity for their application in human therapies. An important characteristic of MSC is their immunomodulatory capacity, which is therefore being explored to treat immune-related diseases. Furthermore, the possibility of MSC being attracted to a site of injury or inflammation due to their chemotactic properties is currently under investigation. It has been already shown that MSC are recruited to an injury site and that some immune cells can attract MSC. In this study, the impact on chemotactic properties and immunomodulation capacity of hMSC expanded in a xeno-free medium was analysed.

Materials and Methods

Bone marrow hMSC were expanded in control medium (DMEM + 10% foetal bovine serum [FBS]) or xeno-free medium. Xeno-free medium is composed of DMEM supplemented with a human plasma-derived material (Supplement for Cell Culture, SCC) developed at Grifols. The ability of monocyte-derived macrophages to recruit hMSC was evaluated by performing migration and invasion assays with transwells.

Immunomodulation was studied by incubating hMSC in combination with a Mixed Leukocyte Reaction (MLR), where there is a co-culture of lymphocytes from different donors (Responder against Stimulator), or by incubating hMSC with resting or Phytohaemagglutinin (PHA) stimulated lymphocytes. Lymphocyte proliferation was measured by using CFSE staining and by analysing the cultures by flow cytometry.

Results and Discussion

Macrophages were able to recruit hMSC cultured in the xeno-free media in both migration and invasion assays, which indicates that xeno-free-expanded hMSC maintained their chemotactic and matrix remodelling properties. Regarding immunomodulation, two different types of results were obtained with the MLR: when proliferation of the Responder cells was higher than 30%, hMSC acted as immunosuppressors; conversely, when the percentage of proliferating cells was lower than 30%, hMSC acted as immunostimulators. In addition, hMSC were able to suppress the proliferation of PHA-stimulated Responder cells. Immunostimulation was observed when co-culturing hMSC with resting Responder cells. Expansion in xeno-free media did not affect immunosuppression or immunostimulation by hMSC.

Conclusions

Xeno-free-expanded hMSC presented the same chemotactic and immunomodulatory properties than hMSC expanded in the commonly used media. Thus, the xeno-free media used is a potential candidate to culture and expand hMSC for human cell therapies.

Disclosure

This work was funded by the European Union 7th Framework Programme under the Marie Curie Initial Training Programme Network IB2 (MC ITN-EID nº 317052). We thank Hospital Sao Joao for buffy coats and bone marrow samples. The authors are indebted to José María Diez, Rodrigo Gajardo and Salvador Grancha (R&D Bioscience Industrial Group, Grifols).

OP-009 microRNA transfer via stem cell-derived microvesicles for enhanced cardiomyogenesis and angiogenesis

*S. Bobis-Wozowicz[1], K. Kmiotek[1], M. Sekula[2], D. Boruczkowski[3], J. Kolcz[4], Z. Madeja[1], E. Zuba-Surma[1]

[1]Jagiellonian University, Department of Cell Biology, Krakow, Poland
[2]Malopolska Centre of Biotechnology, Krakow, Poland
[3]Polish Stem Cell Bank, Warsaw, Poland
[4]Polish-American Children's Hospital, Department of Pediatric Cardiac Surgery, Krakow, Poland

Objectives

Microvesicles (MVs) are small circular cell membrane fragments shed by virtually all cell types under physiological and pathological conditions. MVs play a very important role in inter-cellular communication, by transferring bioactive cargo to other cells in the form of RNAs, proteins and lipids. In this study we aimed to investigate efficacy of MVs isolated from genetically modified human umbilical cord-derived mesenchymal stem cells (hUC-MSC) and human induced pluripotent stem cells (hiPSC), as carriers of selected proangiogenic and cardiomyogenic miRNAs (miR-1, miR-199a and miR-126) to target cardiac mesenchymal stromal cells (cMSC).

Materials & Methods

hUC-MSCs and hiPSCs were genetically engineered to co-express miR-1, miR-199a or miR-126 and green fluorescent protein (copGFP) by lentiviral transduction. Conditioned media from semi-confluent cell cultures were harvested and subjected to sequential ultracentrifugation (2000g; 100 000g x2) to isolate MVs. RNA and protein levels for integrated transgenes were analyzed in MVs and parental cells by real time qPCR and Western blot methods. Transfer of bioactive cargo by MVs to cMSC was performed by co-incubation of MVs with target cells for 24h. Subsequently, cells were differentiated to cardiac or endothelial lineage. Gene expression changes in recipient cells were measured by real time qPCR.

Results

Genetic modification of hUC-MSC and hiPSC resulted in constitutive and stable expression of selected miRNAs and a marker protein (copGFP) in these cells, which was confirmed by molecular analyses at RNA and protein levels. Moreover, MVs isolated from genetically modified cells were enriched in these miRNAs, as well as contained selected cardiomyogenic (GATA4, NKX2.5) and pro-angiogenic (TIE2, FLK1, ENDOGLIN) transcripts. MVs cargo was efficiently transferred to acceptor cells - cMSC and enhanced their differentiation towards cardiac and endothelial lineages.

Conclusions

In this study we have shown that MVs isolated from genetically modified hiPS and hUC-MSC can be enriched in specific miRNAs, which in turn can epigenetically regulate gene expression changes in target cells and enhance their ability to differentiate into a desired phenotype. Obtained results indicate usefulness of stem cells-derived MVs as potential tools in miRNAs transfer, which can be further exploited in regenerative medicine.

OP-010 Differentiation of adipose-derived mesenchymal stem cells to contractile smooth muscle cells for fibrin-based vascular tissue engineering

A. Mrugalla[1], S. Lau[1], T. Aper[2], A. Haverich[1,2], M. Wilhelmi[1,2], *U. Böer[1]

[1]Hannover Medical School, GMP model lab tissue engineering, Hannover, Germany
[2]Hannover Medical School, Cardio-thoracic, transplant and vascular surgery, Hannover, Germany

Aim

Autologous approaches for vascular tissue engineering are a promising alternative to scaffolds based on decellularized xenogeneic arteries that display an unclear risk for immunological rejections. Vascular scaffolds can be generated from fibrinogen which is the effector protein of the blood clotting cascade, can be easily isolated from peripheral blood and is polymerized to fibrin by the addition of thrombin. However, scaffolds as such display an insufficient stability. In natural arteries stability is conferred by the tunica media consisting of connective tissue fibers and smooth muscle cells (SMC) which cannot be isolated directly from donor tissues. Therefore, we here demonstrate the differentiation of contractile SMC from adipose tissue-derived mesenchymal stem cells (ASC) as a suitable autologous tissue source, their characterization and the reseeding of fibrin gels.

Methods

Stromal vascular fraction was isolated from adipose tissue after collagenase digestion and homogenous ASC were obtained after cultivation for 2-3 passages. Tri lineage differentiation and surface marker expression confirmed their mesenchymal stem cell nature. ASC were treated with Transforming growth factor-beta1 (4 ng/mL), bone morphogenetic protein 4 (2.5 ng/mL) and spingosylphosphorylcholin (1µg/mL) for 4-12 days (diff-ASC). Contractile SMC marker expression was determined by Westernblot and IHC and functionality was assessed by gel contraction assays. Fibrin gels (30 mg/mL fibrinogen) were seeded with diff-ASC and untreated ASC visualized by cell tracker green and assessed for tensile stability.

Results

diff-ASC showed a typical SMC phenotype after 4 days and a maximal expression of smooth muscle myosin heavy chain, smoothelin and alpha smooth muscle actin after 8 days. Contraction of diff-ASC seeded collagen gels was 79.3% whereas gels seeded with untreated ASC did not contract. Fibrin gels seeded with diff-ASC showed fiber-like cell accumulations and had a higher stability than unseeded gels.

Conclusion

It was shown that an autologous approach combining contractile SMC differentiated from ASC and highly concentrated fibrin gels exhibits a promising scaffold for the generation of the medial layer of the arterial wall.

OP-011 Lineage-specific haematopoietic metabolism

*C. Böhme[1,2], C. Billing[1], M. Walker[3], N. Noack[1], T. Pompe[2], D. Niederwieser[1], A. Whetton[3], M. Cross[1]

[1]University of Leipzig, Hematology/Oncology , Leipzig, Germany
[2]University of Leipzig, Institute of Biochemistry, Leipzig, Germany
[3]University of Manchester, Stem Cell Proteomics Laboratory, Manchester, Great Britain

Objectives

Myelopoiesis and erythropoiesis are derived ultimately from a common progenitor, but take place at distinct areas in the marrow with respect to the blood supply. We hypothesise that the metabolic environment plays a role in maintaining this distribution by directing the migration of lineage-specific progenitors and/or by supporting lineage-specific patterns of anabolic metabolism. We are therefore examining the metabolic characteristics and requirements of erythropoiesis and myelopoiesis.

Material and Methods

We have established a system by which metabolite gradients can be maintained in a methylcellulose-based colony forming assay and have investigated the effects of glutamine, glucose and lactate gradients on the distribution of colonies arising from human progenitor cell populations purified from umbilical cord blood. Colonies were examined after 14 days, picked and dissociated for morphological identification of the cell types.

In parallel, we have performed metabolite and mitochondrial proteomic analyses of murine multipotent (FDCPmix) cells induced to undergo either erythroid or myeloid differentiation in order to look for changes in metabolic activity associated with commitment and differentiation along these lineages. Mitochondria were purified by density gradient centrifugation and the proteins analysed by iTRAQ. Metabolite flux was assessed by biochemical and mass spectrometric analyses of media conditioned by the cells.

Results

We found no reproducible effect of glutamine, glucose or lactate on the directional migration of colony-forming cells present in either fresh $CD34^+$ population, or in the $CD34^+$ $CD133^{low}$ population of erythro-myeloid progenitors. However, we did find that the maintenance of 8mM glutamine throughout the medium supported the development of myeloid colonies but not of erythroid colonies, suggesting that high levels of glutamine are specifically inhibitory or damaging to erythroid progenitors under the conditions of the colony assay.

The proteomic analysis revealed lineage specific changes in a number of pathways involved with generating or neutralising oxidative stress. In addition, there was a specific down-regulation of mitochondrial alanine amino transferase in the erythroid lineage. This enzyme normally supports the entry of glutamate as α-ketoglutarate into the TCA cycle by transferring the amino group to pyruvate. Consistent with this, we found FDCPmix cells undergoing erythroid differentiation to release large amounts of glutamate.

Conclusions

We have so far found no evidence for directional migration of haematopoietic progenitors in response to metabolite gradients. However, we do find marked differences in the metabolic requirements and activities of erythropoiesis and myelopoiesis. Among these, lineage-specific shifts in the patterns of glutamine metabolism may be responsible for the selective repression of erythropoiesis in the presence of high glutamine.

OP-012 From study on autologous brain cell transplantation to clinical trial: GMP cell production

*J.- F. Brunet[1]

[1]Centre de production cellulaire, LAC/DL, Epalinges, Switzerland

Restoring function of the central nervous system is a challenging task. The large cell replacement experience has offered promising results with different types of cells. Here we propose the adult brain cell autotransplantation as an attractive restoration alternative.

The strategy of autologous reimplantation was investigated in two monkey models: motor cortex lesion as a model of stroke and MPTP-treated monkeys as a parkinsonian model. Primocultures were obtained from cortical biopsy. Cells were grown *in vitro* as neural cell ecosystem that consist in astrocytes and neural progenitors. In both models the cells survived and were migrated to the affected structures, respectively to the lesion area and the striatal structures. Significant functional recoveries were thus observed in both models.

Based on such preclinical result, a phase I-II clinical trial is planned for application in patients affected by stroke.

Prior to that clinical trial, the cell production has to be performed in GMP conditions. For that purpose, and other applications in cell therapy, our public institution, the Lausanne University hospital, implemented a Cell Production Center (CPC) that is now accredited by Swissmedic (authorization n°507482). At the CPC an original concept with a module for production, Isocell Pro 1.8, was developed and qualified. This equipment concept allows to maintain a hermetically culture production in a class A environment by working in class D for operators.

As we all know, the way is long and costing from research to clinical applications. The Lausanne University hospital found a way to help teams of clinicians and researchers to approach that challenge in regenerative medicine.

OP-013 Inhibition of cyclooxygenase-2 alters wnt/β-catenin signaling in the regenerating tail of lizard *Hemidactylus flaviviridis*

*P. Buch[1], S. Balakrishnan[1]

[1]The M. S. University of Baroda, Department of Zoology, Vadodara, India

Epimorphic regeneration in vertebrates involves the restoration of lost tissue or organs through the formation of a regeneration blastema and occurs through a complex interaction of a number of molecular signaling pathways. Of the many effectors of successful tail regeneration in the lizard *Hemidactylus flaviviridis*, one crucial pathway is the Cyclooxygenase-2 (COX-2) mediated PGE_2 signaling pathway. The current study was aimed at understanding whether COX-2 signaling plays any role in the expression of Wnt/ β-Catenin signaling components during regenerative outgrowth in *H. flaviviridis*.

Lizards were administered a dose of specific COX-2 inhibitor Etoricoxib orally as an aqueous suspension. Etoricoxib assimilation in the tail tip through the stages of regeneration was validated by LC-MS/MS analysis and COX-2 inhibition in the tissue was authenticated using a COX-2 activity assay kit. Following COX-2 inhibition, a western blot analysis of β-Catenin was carried out to check for any changes in its expression versus control during two critical milestones of regeneration - Wound Epithelium and Blastema formation. The expression of Wnt5b was assessed by real time PCR.

Results from our study show that an inhibition of the inducible COX isoform - COX-2 - lead to a reduction in expression of β-Catenin during the wound healing as well as the blastema stages. This could be attributed to the altered expression of Wnt5b, which was found to be reduced in response to Etoricoxib treatment. This study leads us to believe that the retardation of tail regeneration in response to COX-2 inhibition, observed earlier in our lab, could be due, at least in part, to an alteration in the Wnt signal, which is known to be essential for the initiation and progression of regeneration. An understanding of the interaction among various signaling pathways will help elucidate the mechanism underlying epimorphosis in lizards, the only amniotes capable of appendage regeneration.

OP-015 Evaluation of the effect of an injection of mesenchymal stem cells on the musculoskeletal status of dogs operated by tibial plateau leveling for a cranial cruciate ligament rupture

*Q. Cabon[1], M. Febre[2], S. Maddens[2], C. Robert[2], C. Boulocher[3], N. Saulnier[2], Y. Chotar-Vasseur[1], T. Cachon[1], E. Viguier[1]

[1]VetAgro Sup, Surgery Department - Small Animals, Marcy l'Etoile, France
[2]Vetbiobank, Marcy l'Etoile, France
[3]VetAgro Sup, Marcy l'Etoile, France

Question

Cranial cruciate ligament rupture (CCLR) is a common orthopaedic condition in dogs. Instability of the stifle generates cartilage and ligament debris that lead to destructive joint inflammation and osteoarthritis. Surgical treatment of CCLR aims to stabilize the knee to limit osteoarthritis evolution. In addition to surgery, new treatment options to manage cartilage degeneration are explored. Among them, mesenchymal stem cells (MSCs) possess anti-inflammatory properties and a potential chondrogenic differentiation; there are ideal candidates for osteoarthritis treatment. In an induced experimental arthritis model, it has been demonstrated that intra-articular injection of MSCs has no side effect and induces an anti-inflammatory and anti-catabolic effect.

The objective of this study is to evaluate the clinical efficacy of MSCs, for analgesia and anti-inflammatory effect, in comparison with a non-steroidal anti-inflammatory drugs (NSAIDs) in dogs operated for CCLR.

Methods

This study is prospective, randomized, controlled, double-blinded. Dogs presented for CCLR were included in the study. Diagnosis was confirmed arthroscopically and stifles were stabilized by tibial plateau levelling osteotomy (TPLO). MSCs used in the study are allogeneic umbilical canine MSCs. Fourteen dogs (16 stifles) included in the study were randomly assigned into two groups. The group "MSC" received an injection of MSCs at the end of intervention in the operated knee and then received a dietary supplement for 1 month postoperatively. "Control" group received a control injection (saline) at the end of intervention in the operated knee and then received NSAIDs (carprofen) for 1 month postoperatively. Preoperative assessment of dogs consisted in a clinical score by a single evaluator and radiographic examination (osteoarthritis assessment, average of 3 readings by 2 different evaluators). After surgery, a daily clinical evaluation by the same evaluator was conducted during the 3 immediate postoperative days. Dogs underwent a clinical evaluation and radiographic examination at 1 month postoperatively. A single evaluator performed all clinical and radiographic evaluations blindly. Data were statistically compared between the 2 groups (preoperative clinical score at J1, J2, J3 and 1 month, preoperative radiographic grade; Fischer and Mann-Whitney tests).

Results

Fifteen stifles in 14 dogs were included in the study, following the exclusion of one case of postoperative infection. No significant difference in weight ($p = 0.79$), age ($p = 0.42$), gender distribution ($p = 1.0$), clinical score ($p = 0.39$) or osteoarthritis status of the operated knee at admission ($p = 0.25$) was found between the 2 groups.

No significant difference in clinical score was observed between the 2 groups, either on day 1 ($p = 0.64$), day 2 ($p = 0.18$), day 3 ($p = 0.1$) or at 1 month ($p = 0.25$).

Conclusion

No local or systemic side effect was observed after intra-articular injection of allogeneic umbilical MSCs. This injection is clinically well tolerated in dogs.

Postoperative clinical scores appear to be similar between the two groups, for lameness and joint comfort. Intraarticular injection of MSCs could be included in the therapeutic options available for the management of osteoarthritis or perioperative inflammation.

One limitation of this study is the small number of patients in each group. The lack of difference between groups could be secondary to a type II statistical error.

OP-016 MSC recruitment during human immune responses to polylactic acid and chitosan 3D scaffolds

*H. R. Caires[1,2,3], M. Navarro[4], M. A. Barbosa[1,2,3], C. R. Almeida[1,2]

[1]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[2]INEB - Instituto de Engenharia Biomédica, Microenvironments for NEWTherapies, Porto, Portugal
[3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[4]CIMNE - International Center for Numerical Methods in Engineering, Barcelona, Spain

Objective

Implanted biomaterials elicit an inflammatory response, whose delicate balance will determine the effectiveness of tissue repair/regeneration. The events that facilitate the shift from detrimental inflammation to constructive tissue remodeling and regeneration involve orchestrated cell recruitment and modulation of immune system. Mesenchymal Stem/Stromal Cells (MSC) can play an important role in the regenerative process due to their capacity to migrate to damaged tissues, to differentiate in different lineages and to their paracrine and immunomodulatory properties. Here, our goal is to analyse how immune responses triggered by different polylactic acid (PLA) and chitosan 3D scaffolds interfere with bone marrow MSC dynamical behavior.

Materials and Methods

NK cells and monocytes were isolated from healthy donor human buffy coats and cultured on TCPS (tissue culture polystyrene) or in 3D chitosan and PLA scaffolds for 48 hrs. Macrophages were obtained by allowing monocytes to differentiate in the biomaterials for 9 days. Metabolic activity and DNA quantity were accessed with resazurin and Picogreen assays, respectively. Actin and nuclei staining were performed to visualize the cell distribution and morphology in the 3D materials. Conditioned media of macrophages cultured in TCPS, Chitosan or PLA scaffolds were analyzed by protein arrays. For MSC recruitment analysis, 600,000 immune cells were pre-cultured on the materials in DMEM without serum for 24 hrs. Then, an invasion assay was set for another 24 hrs by adding 40,000 human bone marrow MSC in the upper compartment of a matrigel-coated transwell system. Analysis of MSC motility in 3D chitosan scaffolds pre-cultured or not with macrophages was performed using a Dendra2 imaging platform[1].

Results

PLA and chitosan lead to increased metabolic activity of macrophages but not PBMCs, NK cells or monocytes. However, this increase was not correlated with cell number. Protein arrays revealed a panel of cytokines whose expression was stimulated by incubation with each material. Importantly, while both NK cells and monocytes in TCPS lead to high number of recruited MSC, macrophages differentiated in the presence of either chitosan or PLA scaffolds were the most effective in promoting recruitment of MSC. Furthermore, imaging of Dendra2 labeled and photo-converted MSC to characterize their motility in 3D microenvironments revealed 43% more mobility when in co-culture with macrophages over 7 days.

Conclusions

Overall, distinct immune populations responded differently to diverse biomaterials, which impacted on the extent of MSC recruitment. This study provides insights for the development of strategies modulating host responses to attract specific progenitor cells for a constructive remodelling of implanted biomaterials.

References

1 Caires HR, Gomez-Lazaro M, Oliveira CM *et al.* Finding and tracing human MSC in 3D microenvironments with the photoconvertible protein Dendra2. *Sci. Rep.* 5, 10079 (2015).

Acknowledgements

The authors would like to thank Hospital de São João for kindly donating the buffy coats. This work was financed by FEDER funds -Programa Operacional Factores de Competitividade - COMPETE and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia in the framework of project EXPL/BIM-MED/0022/2013.

OP-017 Treatment of osteoarthritis with genetically modified multipotent stromal cells

*A. Cicchetto[1], S. Kalomoiris[1], J. Beegle[1], F. Fierro[1]

[1]University of California, Davis, Cell Biology and Human Anatomy, Sacramento, United States

Osteoarthritis (OA) is a degenerative joint disease estimated to affect 630 million people worldwide. OA is characterized by the progressive loss of articular cartilage, damage to subchondral bone and chronic inflammation; unfortunately, there is no cure for OA. Mesenchymal stem cell/Multipotent Stromal Cells (MSC) have been evaluated as a potential treatment, as these cells can contribute through differentiation into bone and cartilage, and act as trophic mediators to reduce inflammation and promote healing. The safety profile of MSC therapy has been widely demonstrated and currently, at least 13 clinical trials are testing the efficacy of MSC to treat OA.

We hypothesize that the efficacy of MSC therapy can be enhanced using lentiviral vectors to overexpress key factors including interleukin-1 receptor antagonist (IL-1Ra), IL-10, and fibroblast growth factor-2 (FGF-2). Based on our experience on how to perform these modifications in a clinically-compliant manner, our primary goal was to functionally characterize these genetically modified MSC *in vitro*. We show effective over-expression of the respective transgenes by ELISA, address number of viral insertions per cell and demonstrate that MSC over-expressing FGF-2 show increased proliferation and reduced differentiation potential into both the osteogenic and adipogenic lineage. In contrast, over-expression of IL-1RA or IL-10 did not affect cell proliferation and only minimally reduced osteogenic differentiation. Most important, MSC over-expressing IL-10 show immune suppressive abilities *in vitro*, as proliferation of PHA-activated peripheral blood mononuclear cells (PBMCs) were strongly inhibited when co-cultured with MSC-IL-10. Transwell assays show a similar trend, suggesting that MSC over-expressing IL-10 suppress PBMC proliferation through the secretion of soluble factors.

These results support the notion of a "second generation of MSC", using genetic modifications to enhance their therapeutic efficacy, while maintaining their excellent safety profile.

OP-018 Employing miRNA - mRNA interaction analysis to link biologically relevant miRNAs to stem cell identity testing for next-generation culturing development

M. Crabbé [,#], B. Vaes [*]

[*]ReGenesys BVBA, Gaston Geenslaan 1, 3001 Heverlee, Belgium
[#]*funded by an IWT Baekeland research grant*

Objectives

The Multipotent Adult Progenitor Cell (MAPC®) therapeutic benefit has been demonstrated in multiple disease models and is advancing in clinical trials. Robust quality assurance is imperative to make advancements in the culturing procedure to enable large-scale cell manufacturing without hampering therapeutic applications. This requires the identification of cell specific markers that do not only distinguish it from other cell types, but can also be linked to important stem cell functions. miRNAs are shown to be master regulators of biological processes and are potentially ideal quality markers.

We aimed to determine miRNA markers differentially expressed under MAPC and MSC culturing conditions with known functions in stem cell biology. These cell types were selected since both are bone marrow derived stem cells that exert therapeutic functions, but have different proliferative and regenerative capacities making correlation of miRNA and stem cell function possible.

Additionally, expression of the miRNA markers needed to be maintained when culturing MAPC on next-generation culturing platforms, such as xeno-free and 3D bioreactors, in order to verify their use for cell comparability testing.

Materials and Methods

To determine cell specific marker miRNAs and assess their effects on stem cell qualities, a full miRNA (qPCR-based) and mRNA (Array-based) profiling was performed on MAPC and MSC isolated from three shared donors. We applied an IPA (Ingenuity)-based strategy that combined an integrated RNA profile analysis and a biological function analysis to determine the effects of miRNA-mRNA interactions on the phenotype.

To ensure the robustness of the selected miRNA marker panel, we validated the expression of the miRNA markers on xeno-free and 3D-bioreactor expanded MAPC cultures, in addition to standard 2D cultures on plastic.

Cell comparability testing of the next-generation MAPC cultures was performed using flow cytometry, qPCR, *in vitro* angiogenesis assays and T-cell proliferation inhibition assays.

Results

The miRNA-mRNA interaction analysis resulted in the identification of important miRNA markers linked to cell cycle regulation and development. The final panel of 13 miRNA markers correlated strongly to differences between the MAPC and MSC phenotypes, highlighting their functions in stem cells biology. Importantly, miRNA marker expression is maintained in xeno-free and bioreactor isolated and expanded MAPC cultures, shown to harbor the same therapeutic potential as standard MAPC.

Conclusion

In conclusion, by analyzing miRNA-mRNA interactions in MAPC and MSC we identified a panel miRNA markers with a clear link to stem cell related processes. Furthermore we propose that these newly identified biologically relevant miRNA markers can be used during further process development to monitor stability during variations in the culturing procedure of MAPC.

OP-019 A systems biology strategy to direct cell fate determination

*A. del Sol[1], S. Ramachandran[1]

[1]LCSB, Esch-sur-Alzette, Luxembourg

Regenerative medicine is a rapidly growing research area, with the great potential to replace or regenerate damaged or lost human cells. However, current therapies that focus on tissue regeneration are significantly impeded by our limited understanding of how to direct cellular differentiation, especially *in vivo*. Since cellular niches (microenvironment) are determinants of cell fate decisions *in vivo*, regenerative therapies require the understanding of the niche effect on stem cells. Nevertheless, to our knowledge, there are no computational tools considering the role of the niche in differentiation, which could aid experimentalists in systematically designing perturbation strategies for desired cellular differentiation events. To this end, we developed a computational method that identify key signaling pathways that regulate cell fate lineage specifiers, and therefore whose perturbations could induce cellular differentiation. These cell lineage specifiers reside in positive feedback loops, which determine the gene regulatory network stability of progenitor and daughter cells. Our method relies on cell-type specific inference of signalling and gene regulatory networks in an integrated manner to account for the niche effects, and it requires as input only transcriptome data of progenitor and daughter cells. Our predictions included experimentally known and novel combinations of perturbed signalling proteins inducing differentiation of hematopoietic stem cells, cardiac stem cells, and mesenchymal stem cells. In addition, we predicted candidate signalling proteins inducing neural stem cell differentiation into neurons, which have been experimentally validated using combinations of targeted compounds.To our knowledge, this is the first computational method that systematically predicts signaling proteins regulating lineage specifiers of cellular differentiation without prior knowledge of potentially involved pathways. Given the increasing interest of cell fate determination in regenerative medicine and basic research, our method represents a useful computational methodology to assist researchers in the field in designing experimental strategies.

OP-026 Three-dimensional gradient delivery of novel highly efficient gag-binding enhanced transduction proteins for directing cell fate

*H. Eltaher[1,2], J. Yang[1], J. Dixon[1], K. Shakesheff[1]

[1]University of Nottingham, Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), Nottingham, Great Britain
[2]Alexandria University, Department of Pharmaceutics, Faculty of Pharmacy, Alexandria, Great Britain

Objectives

Biological processes occurring within the cellular microenvironment such as migration, angiogenesis and differentiation are guided via spatially dependant signals. Biomimetic approaches aim at replicating this complex three-dimensional (3D) cellular microenvironment by the careful design of the scaffold micro-architecture. From this perspective, the aim of this study was to develop biomimetic stable 3D gradients of novel bioactive agents and to study the corresponding cellular responses in a concentration dependant manner replicating the natural microenvironment.

Methods

A compartmentalized diffusion model of Source-Gel-Sink (So-G-Si) assembly was employed to generate 3D biomolecule gradients across cell-laden hydrogels. We utilized a novel approach developed within our research group, named GAG-binding Enhanced Transduction (GET) that produced a fusion protein comprised of a membrane docking peptide to heparan sulfate glycosaminoglycans (GAG) together with a cell penetrating peptide (CPP) to efficiently deliver proteins. GET proteins were tagged with reporter monomeric red fluorescent protein (mRFP) to track their intracellular activity. Gradients of mRFP and GET-tagged mRFP proteins were created via diffusion through a cell-laden hydrogel scaffold from source to sink compartment and the respective protein concentrations were monitored as a function of time and location inside scaffold at 20 µm resolution by serially slicing the scaffolds perpendicular to the direction of protein diffusion using Leica CM1100 cryostat at -20°C. Alternatively, gradient intracellular transduction as a function of time and location was assessed using a MoFlo™ DP (DAKO) Flow Cytometer

Results

The gradient of GET-mRFP across the hydrogel was well-maintained over time as compared to mRFP which diffused out to equilibrium throughout the hydrogel scaffold. Flow cytometric analysis of the retrieved encapsulated cells following enzymatic/heat digestion of serially sectioned hydrogel slices revealed that the cells acted as sink that retained the GET-mRFP and maintained a stable 3D gradient across the hydrogel with gradual cellular responses as a function of distance away from source protein.

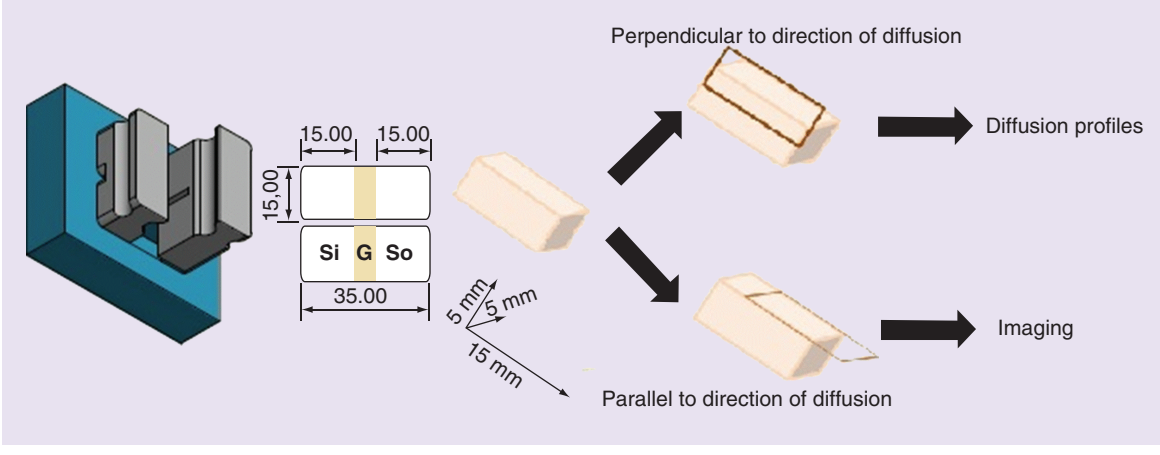


Figure 1. Method outline showing compartmentalized diffusion chamber

OP-026

Conclusions

The ability to spatiotemporally control the intracellular delivery of functional bioactive proteins produced via GET technology in 3D gradient manner will be a powerful tool for directing cellular behaviour and controlling stem cell differentiation in 3D environments.

Acknowledgements

Thanks to Dr. David Onion (Flow cytometry facility, university of Nottingham) for helpful discussions and the Ministry of Higher Education in Egypt for the PhD scholarship.

OP-027 The European project REBORNE: multipotent mesenchymal stromal cells and biomaterial for bone healing

*A. Erle[1], R. Lotfi[1], H. Schrezenmeier[1]

[1]Ulm University Hospital, Institute of Transfusion Medicine, Ulm, Germany

The European project REBORNE targets healing of long non-union bone fractures by application of human bone marrow-derived Multipotent Mesenchymal Stromal Cells (BMMSC) and MBCP+ granules as biomaterial. 23 institutions, among them 12 clinical centers, in 8 European countries are involved. We have established and published one-step as well as two-step large-scale protocols for GMP-compliant *ex vivo* expansion of BMMSC, using pooled human platelet lysate as cell culture growth supplement. Our Advanced Therapy Medicinal Product has been characterized extensively by flow cytometry, cytokine/chemokine production, and differentiation capacity. Its potency for bone healing has been shown in animal models, including mouse, rabbit, sheep, and pig. 4 clinical studies are ongoing or completed. Our contribution will cover main characteristics of REBORNE: large-scale clinical-grade MSC production, differentiation capacity, safety, animal models, and preliminary results of the clinical trials. This project is funded by the 7th Framework Programme of the European Commission.

OP-028 Cell-based therapies in point-of-care settings: innovative regenerative medicine with missing legal (ATMP) control

*T. Faltus[1], R. Schulz[1,2]

[1]TRM Leipzig, Leipzig, Germany
[2]Centre for Biotechnology and Biomedicine, Leipzig University, Leipzig, Germany

Objectives

The project focuses on the legal handling of cell based therapies in point-of-care (PoC) settings in the field of musculoskeletal regeneration. PoC is a process of collecting, processing and administering cells within one medical intervention. There are already various medical devices and treatment options for cell based PoC concepts available and published. Cell based PoC concepts are considered to have a lot of benefits, e.g. minimizing surgery-related burden for the patient, being rapid and/or economic. However, the regulatory (ATMP) framework for PoC cell based therapies leaves numerous loopholes which open the door for unsafe, inefficient, ineffectiveness or unproven cell therapies. Additionally, the interpretation of the pharmaceutical or rather regulatory terms "substantial manipulation" and "same essential function" remains unclear.

Material and Methods

The project evaluates the current state of the art in cell based PoC therapy models and analyses the legal handling of such therapy concepts in an interdisciplinary approach. The project extrapolates the current technical development to examine technical advancements of such therapy concepts in the future. Finally, the projects examines within a technological impact assessment how such prospective therapies would be regulated by the current European pharmaceutical legislation and its implementation into German law.

Results

The legal handling of cell based therapies in PoC settings is incomplete. Depending on the processing of the biopsied cells only in some cases a manufacturing licence according to pharmaceutical law is mandatory. The lack of regulation also causes that some of the PoC therapies do not have to be manufactured under GMP standard. Therefore, in many settings such therapies are either completely unregulated or only partly regulated although the used cells have the character of a cell based pharmaceuticals comparable to other regulated cell based ATMP therapies. Additionally, for cell therapies in PoC settings there is no legal need to proof such therapies by quality studies, non-clinical, and clinical trials.

Conclusions

The assurance of safe, efficient, and proven cell therapies in PoC settings needs a legal (ATMP) framework by the EC/national competent authorities which includes all therapy concepts where cells are used for therapy purposes. The major obstacle here is the separation of medical device law and pharmaceutical (ATMP) law. The project proposes changes in legislation where the current pharmaceutical legislation seems to be insufficient to ensure safe and efficient treatments for cell therapies in PoC settings.

OP-029 Human embryonic stem cell based therapies: no patent, no therapy for regenerative medicine

*T. Faltus[1,2]

[1]TRM Leipzig, Leipzig, Germany
[2]Martin Luther University Halle-Wittenberg, Faculty of Law, Economics and Business, Halle an der Saale, Germany

Objectives

In October 2011 the European Court of Justice (CJEU) decided in the case of *Brüstle v Greenpeace* that patent claims encompassing human embryonic stem cells (hESC) were patent ineligible in the European Union (EU) on public order and morality grounds. In a subsequent case *International Stem Cell (ISCO) Corporation v Comptroller General of Patents, Designs and Trademarks (UK)* of December 2014 the CJEU specified that patent claims encompassing hESC obtained from unfertilised human cellular entities whose development have been stimulated by parthenogenesis are patent eligible since such entities do not have the inherent capacity of developing into a human being. In the aftermath of the Brüstle ruling a rash of stories has appeared predicting the exodus of hESC research. The ISCO ruling therefore has been deemed to give a legal and actual chance to the development of therapies based on hESC. Irrespective of whether these predictions are justified, it is legally still unclarified whether these decisions have an implication on the justification of hESC based therapies. Therefore, this project presents results about the logical link between these patent rulings and the justification of therapies based on hESC in respect to their official market approval.

Material and Methods

First, the project has analysed the patent rulings of the CJEU and investigated whether there is a technical link between the nature of the official market approval and the official patent granting. Second, the project connects the CJEU patent rulings to patent decision within the European Patent Organisation (EPO) dealing with hESC.

Results

Market approval for therapies based on hESC by European regulators has to be linked to the CJEU's decision in the Brüstle and ISCO case since both legal questions - market approval and patent eligibility - are governed by law of the same lawmaker, namely, the EU, and refer to the same moral question, namely, the use of human embryos for the benefits of others. Therefore, it rather seems more consistent to expect the same moral standards in patent and pharmaceutical law. Furthermore, as long as inventions based on hESC obtained from human embryos (in the meaning of patent law) cannot be patented because this is classified as a violation of the public order in terms of a violation of fundamental principles safeguarding the dignity and integrity of a person, namely, the embryo, the same must be true for pharmaceuticals based on hESC. Such pharmaceuticals may not be approved for the market — at least not as long as the safeguarding of the dignity and integrity of a person legally starts just with being a totipotent entity. Finally, this finding is irrespective of whether or not the embryo has to be destroyed or not in order to obtain the hESC.

Conclusions

The EU and national lawmakers are called to clarify whether therapies based on hESC are in compliance with current statutes or not. If such therapies are not in compliance with current law or if they will be prohibited, lawmakers must find a justification for this. However, if a specific therapy based on hESC gets market approval before the aforementioned clarification, it would a priori not be impossible that there would be attempts by a third party to get a judicial review up and running — potently again at the CJEU. In that case, it would be amazing if the same court could have a different moral opinion on the purposeful destruction of embryos than it had before in patent law.

OP-030 A new preclinical model of bone remodeling around titanium implants in rat tail vertebrae

*S. Farkasdi[1], G. Hriczó-Koperdak[1], R. Rácz[1], T. Harangozó[1], S. Koncz[1], B. Kerémi[1], J. Blazsek[1], D. Pammer[2], B. Szabó[3], C. Dobó-Nagy[3], F. Cuisinier[4], G. Wu[5], G. Varga[1]

[1]Semmelweis University, Department of Oral Biology, Budapest, Hungary
[2]Budapest Technical University, Department of Materials, Science and Engineering, Budapest, Hungary
[3]Semmelweis University, Department of Oral Diagnostics, Budapest, Hungary
[4]Université de Montpellier 1, Laboratoire de Biologie Santé et Nanoscience, Montpellier, France
[5]Academic Centre for Dentistry Amsterdam (ACTA), Department of Oral Cell Biology, Amsterdam, Netherlands

Objectives

Replacement of the lost part of the body, as well as bone regeneration, is widely used in medicine. Indeed, functional tooth replacement and bone regeneration are in the daily practice of modern dentistry. The constant flow of innovations in bone grafting biomaterials and titanium surface functionalization justified numerous preclinical studies. We aimed to develop a new animal model to evaluate bone regeneration and osseointegration of dental titanium implants. We used rat tail vertebrae because of their great similarities in bone architecture to the mandible.

Materials and methods

Female Wistar rats (Crl(Wi)Br, Charles River; 250-370 g) were used for our experiments (ethical permission No: 1799/003/2009). Customized titanium implants sized to the rat tail (diameter 2.9 mm coronally and 1.3 mm apically, length 11.38 mm) were used. For implant placement we used a special guided approach at the level of the C4-C5 vertebrae. Validation of the bone remodeling around the titanium implants was performed at 4, 8, 12 and 16 weeks after surgery. Implant stability was measured using a non-invasive system utilizing resonance frequency analysis (RFA) (Osstell AB, Gothenburg Sweden). Stability was displayed as Implant Stability Quotient (ISQ). The axial removal force of the implant from the vertebra was evaluated with a force measurement system (extraction force with Tenzi, pull-out test (TENZI Ltd., Budapest, Hungary)). The extraction force is expressed in Newtons (N). We also performed micro-CT evaluation. Biomechanical properties during osseointegration were further characterized by histomorphometric analyses and with measuring the maximum extraction force.

Results

The strength of osseointegration increased gradually with time as measured by the force needed to extract the implant. Extraction force to pull out the implant on weeks 4, 8, 12 and 16 were 26.54±2.54N, 56.17±5.0N, 171.75±15.12N and 157.57±41.44N, respectively. Although RFA values also increased numerically by time, their increases were at a much lower extent (on weeks 4, 8, 12 and 16 were 32.84±8.86, 34.67±2.57, 32.30±2.08 and 51.54±1.32 ISQ, respectively). There was a weak correlation between extraction force values and RFA measurements (Spearman, r=0,2092) based on the 38 data points measured both by the pull-out test and the RFA. Histomorphometry and micro-CT results showed the new bone formation on implant surfaces suggesting the development of direct connection between bone and titanium.

Conclusions

Our results provide evidence that the caudal vertebrae implant can be a useful standard model for preclinical evaluation of osseointegration of titanium implants and new bone formation. Based on the outstanding similarities in bone architecture and embryological development of rat caudal vertebrae and mandibular bone, this model may serve for preclinical modeling of surgical validation steps in medical and dental implantology.

Disclosure

Supported by French-Hungarian TET-Balaton Program (TET_12_FR-2-2014-0010); the Hungarian National Research Development and Innovation Office (OTKA-NKTH-CK80928; TAMOP-4.2.1/B-09/1/KMR-2010-0001; TAMOP-4.2.2/B-10/1-2010-0013); Grants of Faculty of Dentistry of Semmelweis University (2013, 2014).

OP-032 Towards a novel alginate-based scaffold system for cardiac tissue engineering

*B. Fischer[1], M. Gepp[1], A. Schulz[1], H. Zimmermann[1], L. Gentile[1]

[1]Fraunhofer IBMT, Medizinische Biotechnologie, Sankt Ingbert, Germany

Objective

Cardiovascular diseases cause one third of all deaths worldwide [1]. This shows the need for a system,which is highly comparable to physiological conditions, while offering high flexibility to meet user's needs.

This work explores the combination of 3D printed alginate scaffolds with human induced pluripotent stem cells (hiPSCs)-derived cardiomyocytes as a disease model for the cardiac tissue. In order to engineer the alginate scaffold, we used the GeSim Bioscaffolder (GeSim, Grosserkmannsdorf, Germany) due to his high flexibility and its ease to use. The alginate scaffold is highly biocompatible, lacks xenobiotics and at the same time, mechanical properties can be adjusted [2]. Also, adhesion promoting extracellular matrix proteins (e.g. fibronectin, collagen, laminin, matrigel) can be covalently bound to the scaffold surface [3], creating an exceptional microenvironment to study the myocardium.

Furthermore, this system has therapeutic potential as biocompatible graft populated with hiPSC-derived patient-specific cardiomyocytes. In this work the effects of physical (i.e. hardness, elasticity, shape, surface-to-volume ratio) and chemical (i.e. coupled adhesion molecules) cues on cell adhesion, differentiation, morphology and functionality are investigated.

Material and methods

Sterile, ultra-high viscosity alginate [4,5] was dispensed via the Bioscaffolder (GeSim). Gelling of hydrogel was achieved via the use of bivalent cations ($BaCl_2$, 20mM), which provides stability and durability [4]. In this work matrigel was used to coat the scaffold and promote cell adhesion. Cor4U cells (hiPSC-dervied cardiomyocytes, Axiogenesis) were inoculated and cultured on matrigel-coated alginate scaffolds.

Results

The alginate scaffolds can be 3D printed and adjusted to create varying three dimensional shapes. Preliminary experiments showed that matrigel-coated alginate scaffolds promote cardiomyocyte adhesion and allow long-term culture, maintaining spontaneous depolarization.

Future experiments will focus on the effect of different scaffold shapes and functionalizations on cardiomyocytes adhesion, contractile strength and frequency, as well as the effects on the differentiation of hiPSCs into cardiomyocytes on the alginate scaffold.

Conclusion

The 3D printing technique used in this work leads to a robust and controllable scaffold creating process. Additionally the possibility to covalently bind different adhesion proteins to the surface offers a highly flexible model system. The excellent adhesion properties, the enhanced contractions and the three dimensional nature of the scaffolds suggest a remarkably physiological and highly adjustable microenvironment compared to conventional two dimensional cell culture. The further development of alginate scaffolds could lead to therapeutic applications in which tissue has to be directly substituted to fully emphasize their potential.

References

1 Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. *Eur. Heart J.* 35(42), 2950–2959 (2014).

2 Malda J, Visser J, Melchels FP *et al.* 25th Anniversary Article: Engineering Hydrogels for Biofabrication. *Adv. Mater.* 25(36), 5011–5028 (2013).

3 Rowley JA, Mooney DJ. Alginate type and RGD density control myoblast phenotype. *J. Biomed. Mater. Res.* 60(2), 217–223 (2002).

4 Zimmermann H, Zimmermann D, Reuss R *et al*. Towards a medically approved technology for alginate-based microcapsules allowing long-term immunoisolated transplantation. *J. Mater. Sci. Mater Med*. 2005. 16(6), 491–501 (2005).

5 Storz H, Müller KJ, Ehrhart F *et al*. Physicochemical features of ultra-high viscosity alginates. *Carbohydr. Res.* 344(8), 985–995 (2009).

OP-033 Specific transcripts of *DNMT3A* modulate differentiation of hematopoietic stem and progenitor cells

*J. Frobel[1], T. Božić[1], A. Raić[1], Q. Lin[2], S. Heilmann-Heimbach[3], A. Hofmann[3], T. Goecke[4], E. Jost[5], W. Wagner[1]

[1]Helmholtz-Institute for Biomedical Engineering – Stem Cell Biology and Cellular Engineering, RWTH Aachen University Medical School, Aachen, Germany
[2]Helmholtz-Institute for Biomedical Engineering – Cell Biology, RWTH Aachen University Medical School, Aachen, Germany
[3]Institute of Human Genetics, Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany
[4]Department of Obstetrics and Gynecology, RWTH Aachen University Hospital, Aachen, Germany
[5]Clinic for Oncology, Hematology, and Stem Cell Transplantation, RWTH Aachen University Medical School, Aachen, Germany

Objective

DNA methyltransferase 3A (DNMT3A) is a *de novo* DNA methyltransferase that is alternatively spliced in a tissue- and disease-specific manner, but the functional relevance of the different transcripts is hardly known. *DNMT3A* is frequently mutated in patients with acute myeloid leukemia (AML) and we have recently demonstrated that effects of these genetic mutations can be mimicked by an epigenetic dysregulation within the *DNMT3A* gene: about 40% of AML patients revealed aberrant DNA hypermethylation which was associated with shorter overall survival. Notably, *DNMT3A* mutations and "epimutations" seem to have impact on the expression of different *DNMT3A* transcripts. In this study we elucidated the functional role of individual *DNMT3A* transcripts in hematopoiesis.

Methods

Single *DNMT3A* transcripts (Tr. 1+3, Tr. 2, and Tr. 4) were knocked down by lentiviral expression of short-hairpin RNAs in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) that were isolated from human cord blood. Knockdown efficiencies of individual transcripts were validated by qRT-PCR. Subsequently, we evaluated the impact on the proliferation (Carboxyfluorescein succinimidyl ester (CFSE) assay), the immunophenotype (CD34 and CD133 expression), and the colony formation potential (CFU assay) of HSPCs. DNA methylation profiles were analyzed with the Illumina 450k BeadChip technology. Furthermore, gene expression profiles were investigated with the Affymetrix GeneChip Human Gene ST 1.0 Array and are currently under analysis.

Results

Knockdown of individual *DNMT3A* transcripts was confirmed with 30% (Tr. 1+3), 45% (Tr. 2), and 67% (Tr. 4) of basal expression. Downregulation of either Tr. 2 or Tr. 4 reduced the proliferation rate of HSPCs significantly (n=3, $p<0.05$). Notably, HSPCs maintained CD34 expression for more cell divisions upon knockdown of Tr. 2 (n=3; $p<0.05$). In CFU assays the downregulation of Tr. 4 resulted in a clear bias towards erythroid colonies. These functional effects might be governed by specific regulation of DNA methylation patterns. In fact, DNA methylation revealed significant differential methylation of several CpG sites, particularly upon knockdown of Tr. 2 and Tr. 1+3 compared to control cells (8,905 and 352 CpGs, respectively; n=3; adjusted p-value<0.05). Notably, various CpG sites in the promoter region of CD34 were significantly hypomethylated in HSPCs with downregulated Tr. 2 and Tr. 1+3. In contrast, knockdown of Tr. 4, which does not exhibit methyltransferase activity, did not reveal any significant changes.

Conclusion

Our results demonstrate that specific *DNMT3A* transcripts have different effects on global DNA methylation patterns and unique regulatory functions during the differentiation process of blood progenitor cells. This may also be relevant for disease progression in AML.

Disclosure

This work was supported by the Else Kröner-Fresenius Stiftung, the German Research Foundation, and the Interdisciplinary Center for Clinical Research within the Faculty of Medicine at the RWTH Aachen University.
RWTH Aachen Medical School has applied for a patent for the analysis of DNAm patterns and splice variants in DNMT3A for the diagnosis of malignant diseases and Wolfgang Wagner is involved in the company Cygenia GmbH that may provide service for this method.

OP-035 Conclusions from case studies: 10 points to consider when developing a cell therapy product

*D. Gershtein[1]

[1]Gsap, Haifa, Israel

Developing a cell therapy product presents a unique set of challenges. The development process should be with a future clinical application in mind. Bridging the gap between the concepts in the cell therapy industry and the therapeutic application in patient care is the key. If your product cannot be translated into the clinics successfully, the tremendous effort and investment put into such product development would not be beneficial to any of the stakeholders. Our experience with several Israel-based Cell Therapy companies, whom we are leading through this pass from an idea into a therapeutic product, made us realize that there are several key factors, which are common to all cell therapies, regardless of the particular cell type or the target disease. These factors should be considered as early as possible in the product development process in a vision of future clinical applications.

Naming a few

Target patient population could have a tremendous impact on product regulatory requirements, the ability to recruit patients for the clinical study, collect clinical safety and efficacy data and to meet study endpoints;

Cell source selection could affect the product efficacy, immune tolerability, complexity of the cell derivation procedure and robustness of the manufacturing process;

Comprehensive characterization of product identity, purity and potency could significantly advance the different development stages; Greater product stability will allow a greater flexibility in production, patient recruitment and product administration. Moreover, it will shape the future company business model, considering scalability, distribution logistics, clinical sites selection and managment.

A case study from a Cell Therapy development process will be presented, discussing these and other key factors.

OP-037 Regulated cellular invasion through tunable proteolytically degradable hydrogels

*K. Goetsch[1], M. Bracher[1], D. Bezuidenhout[1], P. Zilla[1], N. Davies[1]

[1]University of Cape Town, Surgery, Cape Town, South Africa

The use of biodegradable materials for tissue engineering, regeneration and drug release have the advantage of avoiding a permanent and chronic immune response, as well as reducing the potential need for removal surgery. This combined with the ability to regulate specific biodegradable characteristics, make synthetic biodegradable hydrogels ideal for controlling drug release, development of resorbable devices, and improvement of cellular integration. In this study we investigate whether cellular invasion *in vitro* and *in vivo* can be tightly regulated within proteolytically degradable hydrogel scaffolds with the same stiffness through varying the ratios of two differentially degradable crosslinking peptides. This will allow for a further layer of sophistication in the control of one critical parameter in regenerative medicine, namely tissue invasion. This investigation stemmed from previous work where we had shown a marked difference in invasion into hydrogels crosslinked either with PAN-MMP or MMP-9 peptides for both fibroblasts and smooth muscle cells and that these two hydrogels were mechanically similar for both stiffness and mesh size.

Polyethylene glycol (PEG) hydrogels crosslinked with matrix metalloproteinase (MMP) susceptible peptide sequences have been shown to permit cell-controlled invasion. In this study, hydrogels (20 kDa vinyl sulfone derivatized 4-arm PEG) of the same stiffness (≈236 Pa), assessed via oscillatory shear rheometry, polymerised using different ratios of a readily degradable MMP peptide sequence (PAN-MMP - GCREGPQGIWGQERCG) and a MMP peptide with a limited degradation capacity (MMP-9 - GCREKGPRQITERCG). Proteinase K degradation and fluorescamine labelling were used to assess the differences in protease based degradability between the two MMP peptide sequences. For *in vitro* assessment a sprouting assay was used. An *in vivo* subcutaneous rat implant study was also conducted over 28 days within porous polyurethane discs, followed by histology and tissue invasion quantification.

Spheroid sprouting, *in vitro*, increased as the ratio of MMP-9 to PAN-MMP peptide sequences increased; the sprout length decreased accordingly from 141.44% (100% PAN-MMP), 117.37% (75% PAN-MMP, 25% MMP-9), 86.58% (50% PAN-MMP, 50% MMP-9), 39.45% (25% PAN-MMP, 75% MMP-9) and 23.25% (100% MMP-9). For *in vivo* analysis, the percentage cellular invasion was assessed as a percentage of the control (No PEG hydrogel). At 28 days 155.80% (100% PAN-MMP), 78.93% (75% PAN-MMP, 25% MMP-9), 52.11% (50% PAN-MMP, 50% MMP-9), 32.50% (25% PAN-MMP, 75% MMP-9), and 6.26% (MMP-9) was observed. A correlation of $R^2 = 0.9813$ and significance of p in *in vitro* spheroid sprouting and R2 = 0.9037 and significance of p for *in vivo* tissue invasion reveal a similar invasive response linked to the MMP ratio utilized.

The crosslinking of PEG hydrogels with varying combinations of two peptides with marked differences in proteolytic degradability allowed for the fine titration of both *in vitro* cellular invasion and tissue invasion *in vivo*. This regulation could be achieved without altering the stiffness of the hydrogel. This approach is thus a simple method to control the durability of implanted hydrogels both in the context of encapsulated cells and their replacement by invading host tissue.

OP-038 Epigenetic biomarker to support classification into pluripotent and non-pluripotent cells

*R. Goetzke[1], M. Lenz[2,3,4], A. Schenk[2,5], C. Schubert[6], H. Hemeda[7,1], S. Koschmieder[6], M. Zenke[1,3], A. Schuppert[2,4,5], W. Wagner[1]

[1]Helmholtz-Institute for Biomedical Engineering, Stem Cell Biology and Cellular Engineering, Aachen, Germany
[2]Joint Research Center for Computational Biomedicine, Aachen, Germany
[3]Helmholtz-Institute for Biomedical Engineering, Cell Biology, Aachen, Germany
[4]Aachen Institute for Advanced Study in Computational Engineering Science (AICES), Aachen, Germany
[5]Bayer Technology Services GmbH, Leverkusen, Germany
[6]RWTH Aachen University Medical School, Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Aachen, Germany
[7]PL BioScience GmbH, Aachen, Germany

Objectives

Several methods can be used as surrogate assay to determine pluripotency of induced pluripotent stem cells (iPSCs) - e.g. analysis of iPSC-colony morphology, surface marker expression, analysis of individual genes or gene expression profiles, multilineage differentiation potential *in vitro*, or teratoma formation. There is a clear trade-off between cost- and labor-intensive methods on the one hand and reliability on the other. Differentiation potential of iPSCs is also reflected by a unique epigenetic makeup in DNA methylation (DNAm) profiles. Here we describe a simple method to estimate pluripotency which is based on the DNAm level at only three CpG sites.

Material and Methods

Two of the CpG sites were selected based on their discriminatory power in 258 DNAm profiles (63 pluripotent, 195 non-pluripotent; 450k Illumina BeadChips, www.ncbi.nlm.nih.gov/geo/). They become either methylated or demethylated in iPSCs and their combination is referred to as "Epi-Pluri-Score". In addition, a third CpG located in the pluripotency-associated gene *POU5F1* (OCT4) was considered. This epigenetic signature was validated on independent DNAm datasets (264 pluripotent and 1951 non-pluripotent samples; 27k Illumina BeadChips) with 99.9% specificity and 98.9% sensitivity. Subsequently, we established pyrosequencing assays to specifically analyze DNAm at the CpGs of the Epi-Pluri-Score.

Results and Conclusions

Epi-Pluri-Score analysis allowed reliable classification of 18 pluripotent cell lines and 31 non-pluripotent cell lines. DNAm changes at these three CpGs were subsequently analyzed in the course of differentiation of iPSCs towards mesenchymal stromal cells demonstrating that particularly the CpG site in *POU5F1* demarcates early differentiation events. Notably, the method could also discriminate partially or improperly reprogrammed cells. Taken together, the DNAm level of three specific CpG sites provides a simple and robust biomarker for analysis of pluripotency with high sensitivity and specificity.

This work was supported by the StemCellFactory consortium, co-funded by the European Union (European Regional Development Fund - Investing in your future) and German Federal State of North Rhine-Westphalia (M.Z., W.W., M.L., An. S.); by the German Research Foundation (W.W.:WA 1706/3-2 andWA 1706/2-1), Bayer Technology Services GmbH (M.L., Ar. S., An. S.), and by the Else Kro¨ner-Fresenius-Stiftung (W.W.).

Conflict of Interest Statement

R.G. and W.W. are involved in the company Cygenia GmbH (www.cygenia.com) that may provide service for this method to other researchers.

OP-040 Treatment of advanced gastrointestinal cancer in a clinical phase I/II trial with genetically modified mesenchymal stem cells: A Phase I clinical study

*C. Günther[1], H. Nies[2], J. C. von Einem[3], F. Hermann[4], V. Scherhammer[5], V. Heinemann[3]

[1]apceth GmbH & Co. KG, CEO, Munich, Germany
[2]Hospital of the University of Munich, Department of General, Visceral, Transplantation, Vascular and Thoracic Surgery, Munich, Germany
[3]Hospital of the University of Munich, Department of Medical Oncology and Comprehensive Cancer Center, Munich, Germany
[4]apceth GmbH & Co. KG, Preclinical development, Munich, Germany
[5]apceth GmbH & Co. KG, Clinical development, Munich, Germany

Question

Targeting therapy to cancer and other diseases of high medical need has been a long held goal and a challenge. A large body of published literature, however, points to the unique ability of MSCs to actively home to tumors, areas of inflammation and tissue damage. Here, we describe the development of genetically modified MSCs (gmMSCs) with the inherent ability to target tumors and express any desired therapeutic transgene *in situ*. MSCs might add an additional modality in oncology to target the tumor microenvironment and stroma, independent of specific molecular pathways and mutations. Agenmestencel-T is an autologous cell product, based on bone marrow-derived MSCs, incorporating the Herpes Simplex Virus Thymidine Kinase (HSV-TK) gene, under the RANTES (CCL5) promoter. The cells are administered i.v. and home into tumors, where the RANTES promoter is activated, resulting in the expression of HSV-TK. Ganciclovir (GCV) is then administered i.v. and is activated in tumors by HSV-TK, killing cells in the vicinity via gap-junctions (bystander killing).

Methods

A Phase I/II clinical trial (TREAT-ME 1) was designed and commenced based on *in vivo* efficacy data and proof of concept previously described in mice.

In the Phase I part (completed), six advanced-stage gastrointestinal adenocarcinoma patients were treated (three colorectal, two pancreatic, one cholangiocellular carcinoma). The treatment schedule was an administration of a low (3 patients; $0.5x10^6$ cells/kg body weight/weekly infusion) or a high (3 patients; 10^6 cells/kg body weight/weekly infusion) dose per week, for three weeks, each followed by GCV administration on the 3rd, 4th and 5th day. All protocols were approved by a Data Safety Monitoring Board, a local Ethics Committee and the Paul-Ehrlich Institute.

Results

The treatment was safe and tolerable in all patients. No related Serious Adverse Events or other Adverse Events with CTC-AE Grade 3-5 toxicity were recorded. Patient monitoring by laboratory parameters, cardiac monitoring and vital signs revealed no signs for clinically significant negative changes and trends. Preliminary results also indicate that elevated liver enzymes and cholestasis parameters due to the liver involvement (G-GT, aP, Bilirubin, GPT, GOT) declined significantly in chronological correlation to the therapy. This effect was not sustained after end of treatment and might require repeated doses. According to RECIST (1.1) 4/6 patients showed stable disease at three months follow-up, 2/6 progressive disease. 1/6 was in sustained SD (>5 months). 2/6 patients had stable clinical condition.

Conclusion

This is the first reported clinical trial with gmMSCs and the first report of MSCs being used in oncology. The data support the hypothesis that gmMSCs are a viable, safe and promising therapeutic modality and are consistent with previous observations in mice, where recruitment of cells to the tumors, transgene expression and a significant decrease in tumor volume were seen. Based on the positive data and regulatory approvals, the trial has now entered the Phase II part (open label), to include ten patients and evaluate the safety, tolerability of Agenmestencel-T and its efficacy (RECIST criteria) and establish proof of concept in these patients. Also, a new Phase I trial will commence in 2015 to evaluate the use of donor-derived allogeneic gmMSCs (Agenmestencel-L) for solid tumor treatment.

Week	**1**							**2**							**3**				
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Drug	MSC		GCV					MSC		GCV					MSC		GCV		

OP-041 Examination of host specific immune response after infection with fastidious human pathogens in a 3D *in vitro* model of the human respiratory mucosa

*S. Häusner[1], K. Seidensticker[1], S. Schneider- Schaulies[2], R. Gross[3], H. Walles[1], M. Steinke[1]

[1]Fraunhofer IGB, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Translational Center "Regenerative Therapies for Oncology and Musculoskeletal Diseases" – Wuerzburg branch, Würzburg, Germany
[2]Institute of Virology and Immunobiology of the University of Wuerzburg, Würzburg, Germany
[3]University of Würzburg, Microbiology, Würzburg, Germany

Purpose

Three dimensional (3D) tissue-engineered human tissue models are of high relevance, e.g. to investigate virulence mechanisms of human obligate pathogens *in vitro*. Two major obligate agents causing acute respiratory diseases are measles virus (mv) and *Bordetella pertussis* (Bp), the agent of whooping cough. The progress towards elimination of mv and Bp has stalled which is mainly caused due to an absence of suitable models to gain more knowledge about their pathomechanisms and host cell response. Therefore we are nowadays confronted with a lack of proper vaccination coverage and new research findings in both cases.

Methods

On a biological collagen matrix (SISser) a co-culture of human fibroblasts and either immortalized human airway epithelial cells (Calu- 3, for mv studies) or primary human airway epithelial cells (hTEC, for Bp studies) was seeded and cultured under airlift conditions. The differentiated mv test systems were added with human dendritic cells (hdcs,), infected with the measles virus for 14 days, and afterwards analyzed using light and scanning electron microscopy (SEM). The differentiated Bp test systems were treated with sterile-filtrated supernatants of Bp and afterwards analyzed with transmission electron microscopy. 2D cultures of both hTEC and Calu-3 were also infected with Bp and a GFP linked mv, respectively and investigated using Raman spectroscopy and SEM.

Results

Both 3D test systems of the human airway mucosa show high *in vitro - in vivo* - correlation on both structural and ultrastructural level. Preliminary morphological analysis after infection with Bp/ mv reveals considerable ultrastructural changes which were not observed in control samples. The high-resulted ultrastructural sem -pictures show a clear inauguration of the infected hdcs, comparatively to the *in vivo* situation. In 2D culture conditions the Raman spectra of infected hTEC clearly differ from spectra of the control group.

Conclusion

Our data show that the 3D airway mucosa model represents pathological effects of Bp toxins and offers an opportunity to further examine pathomechanisms and host cell response. The tri- culture- model showed a clear incorporation of the infected hdcs, wherefore the next stage towards a mv model can be achieved. Raman spectroscopy appears to be a practical non-invasive method to show intracellular changes on living cells after infection.

OP-042 Tooth-derived stem cell culturing on poly(asparatic acid) based hydrogels

*O. Hegedus[1], D. Juriga[2], K. Nagy[1], A. Jedlovszky-Hajdu[2], M. Zrinyi[2], G. Varga[1]

[1]Semmelweis University, Department of Oral Biology, Budapest, Hungary
[2]Semmelweis University, Department of Biophysics and Radiation Biology, Budapest, Hungary

Objectives

In the past few years stem cells were successfully isolated from tooth associated tissues, which have multi-differentiation potential and immunomodulatory effects. Conditions supporting the *in vitro* 3D proliferation and differentiation of these cells may increase their applicability in clinical processes. Amino acid-based hydrogels could mimic the properties of the native extracellular matrix (ECM) and provide optimal conditions for the cells. Our aim is to analyze dental pulp (DPSC), and periodontal ligament (PDLSC) derived stem cell culture viability, morphology, proliferation and migration ability on various poly(asparatic acid) based hydrogels (Zrinyi et al, Acta Biomater. 2013; 9:5122) with different physico-chemical properties. Our long-term goal is to tailor these properties to find the exquisite composition, which may subsequently be used in regenerative therapy.

Materials and methods

Cells are originated from impacted human wisdom teeth according to our previously published protocols (*Kadar et al., Journal of Physiology and Pharmacology, 2009. 60(Suppl 7): p. 167-175.*). The cells were seeded on the PASP (polyaspartic acid) based hydrogels. We examined 21 different gels that have different mechanical properties and contain different amino-acid cross-linkers such as diaminobutan (DAB), cystamin (CYS) and lysine (LYS) and some of them also contain different amounts of thiol groups. The gels were pretreated with stem cell culture medium and were sterilized before the seeding. We used 5 internal and at least 4 biological parallels in each experiment. The morphology of the cells was examined by phase-contrast microscope for 7 days. Proliferation and migration kinetics were monitored by videomicroscopy for 24 hours. To visualize the cells growing into the gels, they were labeled with the fluorescent vital dye Vybrant DiD. The examination was carried out with two-photon microscopy on the second and the 4th day. For analyzing cell viability, we used a mitochondrial dehydrogenase activity-based method applying WST-1 reagent, on the second and the 4th day after plating.

Results

While our cells are able to attach and grow on DAB and CYS cross-linked PASP-based hydrogels, they were unable to do so on LYS cross-linked gels. The attached cells show healthy, fibroblast-like morphology. DPSC and PDLSC cells were viable on DAB and CYS gels, while we cannot find viable cells on the LYS cross-linked gels. The highest population of viable cells could be observed when cultivating them on PASP gels containing thiol groups. The increase of the amount of thiol-groups in the gels had a significant positive effect on the adhesion and the proliferation of the DPSC and PDLSC cells. We found that the increase of the hardness of the gel also increased the adhesion and the proliferation of the cells. Phase-contrast and two-photon microscopic analysis also confirmed these results and showed that these cells were able to grow inside the gel matrix in the case of DAB and CYS cross-linked gels.

Conclusions

The thiol-containing PASP gels is found to be most suitable for culturing PDL and DP originated stem cell cultures, since they ensure the conditions for adhering, reproduction and migration. These gels would be good candidates as scaffolds in stem cell-based tissue engineering.

Disclosure

Supported by the Hungarian National Research Development and Innovation Office (TÁMOP-4.2.1/B-09/1/KMR-2010-0001, TÁMOP-4.2.2/B-10/1-2010-0013 and OTKA-NKTH CK-80928).

OP-043 Histological analysis of bone regeneration with different doses of rhBMP-2 in an ovine lumbar interbody fusion model

*K. Siegrist[1,2], J. Seeger[2], H. J. Meisel[3,1], *C. Hohaus[3,1]

[1]Translational Centre for Regenerative Medicine, Leipzig, Germany
[2]University of Leipzig, Faculty of Veterinary Medicine Medical , Institute of Anatomy, Histology and Embryology, Leipzig, Germany
[3]Professional association hospital Bergmannstrost, Dept. Neurosurgery, Halle, Germany

Methods

In this study 22 sheep underwent two level lumbar interbody fusion using an ventrolateral approach with secondary dorsal fixation at L1/2 and L3/4. In one level specially designed PEEK-cages filled with one of three doses rhBMP-2 (total dose 0,5mg; 1mg; 2mg) delivered on an ACS, were implanted. The other level either received an empty PEEK-cage or a PEEK-cage with ACS. The decision in which level the growth factor was implanted was made randomly.Animals were sacrificed after 3 and 6 months and decalcified histology was performed. This included histomorphological analysis as well as histomorphometry of the tissues within the cage.

Results

At 3 months after surgery the groups treated with rhBMP-2 showed higher amounts of bone tissue within the cage, with the 1mg- and 2mg rhBMP-2 groups showing the highest and comparable amounts. At 6 months the amounts of bone tissue increased in all groups, but were still lower in the groups without growth factor. The well known dose-dependent effect of rhBMP-2 on bone healing was also recognized in our study. The highest rhBMP-2 dose (2mg), compared to the lowest dose (0,5mg), produced a higher amount of bone tissue at each investigation point

At 3 months there was only one active osteolysis in the cage/ACS-group with osteoclasts resorbing the bone of the vertebral body. Remarkably, at 3 months 7 of 8 segments of the rhBMP-2 groups had a compromised bone structure around the implant. These areas were filled with fibrous tissue and fibrocartilage. In the periphery active osteoblasts were seen. This finding was not detected in the groups without rhBMP-2 at 3 months. Attention has also to be payed for two segments, one of the lowest and the highest rhBMP-2 dose (0,5mg and 2mg), in which an inflammation with granulomas and giant cells was found at 3 months. Such a strong inflammation was not found in the groups without growth factor. But on the other hand, at 6 months most of the segments with an empty cage or cage/ACS showed a moderate chronic inflammation. Predominant cells were macrophages and giant cells. The groups treated with rhBMP-2 showed only a few mild chronic inflammatory reactions at 6 months. Interestingly the predominant cells were the same. In all groups, macrophages and giant cells were found in fibrous tissue adjacent to the implant. Noticeable, at 6 months all segments without rhBMP-2 that showed a chronic inflammation also had a compromised bone structure adjacent to the cage.

Discussion

The well-known dose dependent effect of rhBMP-2 on bone healing could also be recognized in our study. Attention has to be payed for the proinflammatory properties of the growth factor. Consistent with other studies we found 2 strong inflammatory reactions, each one in the lowest and highest dose group. Also the potential for causing transient bone resorptions, according to the results of others, was demonstrated. At 3 months 7 of 8 segments treated with rhBMP-2 showed compromised peri-implant bone. Osteoblasts, but not osteoclasts, were seen in the periphery of these areas. It can be concluded that there where bone resorptions which already merged into an increased osteoblastic activity. Usually resorptions occur between 2 and 12 weeks and are followed by a period of increased osteoblastic activity. This finding wasn't recognized at 6 months anymore.

Striking is that at 6 months most of the segments without rhBMP-2 showed a compromised bone structure around the implant with a mild to mainly moderate chronic inflammatory reaction. This cannot be attributed to the growth factor. Also the ACS is degraded at 6 months and is unlikely a possible explanation. Therefore, the cage as a reason must be considered and it has to be questioned whether PEEK is the optimal material for interbody cages.

OP-045 EktoTherix™ tissue repair scaffold: translation from test rig to clinIC

M. Raxworthy[1], *P. Iddon[1], L. P. Serino[1]

[1]Neotherix Ltd, York, Great Britain

Objective

EktoTherix™ is a novel 3D bioresorbable polymer scaffold for soft tissue regeneration and repair developed by Neotherix. The collection of significant amounts of performance, safety and manufacturing data for this electrospun poly(glycolic acid) (PGA) scaffold regenerative device has been required to allow progression to full translation of the product. In this presentation, we describe the pathway travelled and the learning gained by Neotherix as a Medical Technology start-up.

Materials and Methods

EktoTherix PGA scaffolds with a mean fibre diameter of 2.5 µm and porosity of 84% were prepared by electrospinning using detailed methods described in international patent application WO 2013/050429 [1].

Performance data obtained *in vitro* (cell adhesion, proliferation and migration; production of ECM components) was verified *in vivo* using a porcine excisional wound model. Wound bed integration and cosmesis (quality of repair) were also assessed and this information was presented at the World Conference on Regenerative Medicine in 2013 [2]. Such performance measurements were necessary to justify progression to validation of concept and clinical opportunity and the development of a controlled, reproducible electrospinning manufacturing process operating within a quality management system. The steps required to produce EktoTherix as a gamma sterilised, single-packed 35 x 35 mm scaffold product, regulated as a medical device will be described with recommendations for other potential developers of biomaterial-based Class III products.

Results

Suitability for translation to the clinic and the market as a treatment for surgical excision wounds (such as those resulting from the removal of skin cancers) is currently being assessed through a First in Man clinical investigation. EktoTherix is designed to provide healthy cells from the edges of a wound with an array of ultra-fine fibres along which to migrate and re-populate the wound space, enhancing repair and reducing the need for skin grafts or skin flaps (which may lead to additional patient morbidity). Pre-clinical evidence indicates that bioresorption of the polymer starts to occur after a few days of application and proceeds to completion within four to six weeks and that highly proliferative and viable cells start to populate and replace the scaffold within the first four days. The design of the Clinical Investigation and preliminary clinical data will be presented.

Conclusions

Pre-clinical porcine wound model data provided evidence that the use of EktoTherix may result in improved wound cosmesis and also provided proof of concept for the use of EktoTherix in excisional wound repair. Learning gained from assembling the evidence and support needed to bring the product to a subsequent clinical trial will be reviewed as a case study for similar medical technology product developments. Further steps needed to commercialise EktoTherix will be discussed in order to ensure all learning from bench to bedside is captured.

Disclosure

This work was supported by grants from Innovate UK and the National Institute of Health Research, UK. We acknowledge the contribution of Lorien Engineering Solutions Ltd and Smith & Nephew Advanced Wound Management as project partners.

References

1 Neotherix Ltd. International patent application WO 2013/050429 (2013).

2 Serino LP, Iddon P, Raxworthy M. *Regen. Med.* 8(6s), S98 (2013).

OP-046 Decellularized precision cut lung slices for studies of repair and regeneration of functional distal lung tissue

*O. Johansson[1], A.- K. Larsson Callerfelt[1], L. Eriksson[1], O. Hallgren[1,2], G. Westergren-Thorsson[1]

[1]Lund University, Lung Biology, Department of Experimental Medical Sciences, Lund, Sweden
[2]Lund University, Respiratory Medicine and Allergology, Department of Clinical Sciences Lund, Lund, Sweden

Objective

Lung transplantation is the only treatment option for many patients with advanced lung disease, but the availability of donor organs is limited. Decellularized lung scaffolds repopulated with cells from the organ recipient is a new potential source of donor lungs. Precision cut lung slices (PCLS) is an established experimental platform for tissue culture studies were complex physiological processes can be studied under cell culture like conditions. We aim to establish an efficient method to produce acellular PCLS scaffolds from patient material for studies of the interaction between cells and extracellular matrix.

Material and methods

Cryopreserved human parenchymal lung tissue were cryosectioned into PCLS and decellularized using an adaptation of a previously described combined detergent and enzymatic decellularization protocol [1]. Decellularization was evaluated with histology as well as with quantitative measurements of DNA and phospholipids. Glycosaminoglycan (GAG) content was evaluated both quantitatively and qualitatively by an in-house HPLC method. The protocol have also been tested on mouse and porcine lung tissue with consistent results.

Results

Our method produces morphologically intact decellularized PCLS with a well preserved alveolar 3D architecture. Efficient decellularization have been achieved with DNA removal down to <5 pg/mg tissue dry weight and a 95% removal of phosphatidylcholine containing phospholipids. The decellularization protocol introduces qualitative changes in the GAG composition of the tissue, the functional importance of these changes have not yet been evaluated.

Conclusions

We have developed a protocol which allow us to produce a large number of decellularized lung tissue slices from small pieces of lung tissue for high throughput studies of the conditions and processes necessary for successful regeneration of functional distal lung tissue.

Decellularized PCLS provides a versatile experimental platform for studies of regeneration in distal lung tissue. Our model combines the potential for high throughput pharmacological studies combined with an efficient use of valuable patient material.

Disclosures

This work was supported by grants from the Swedish Heart-Lung Foundation and the Swedish Research Council.
The authors declare no conflicts of interest.

Reference

1 Sun H, Calle E, Chen X *et al.* Fibroblast engraftment in the decellularized mouse lung occurs via a β1-integrin-dependent, FAK-dependent pathway that is mediated by ERK and opposed by AKT. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306, L463–L475 (2014).

OP-048 *In vitro* creation of vascular structures by laser-assisted bioprinting

*O. Kerouredan[1,2], M. Remy[1], N. Thebaud[1,2], J. Kalisky[1], E. Pages[1], J. Amedee[1], J.- C. Fricain[1,2], S. Catros[1,2], R. Devillard[1,2]

[1]INSERM U1026 (BioTis), BORDEAUX, France
[2]University Bordeaux, Faculty of Dentistry, BORDEAUX, France

Question

Development of microvasculature and microcirculation is critical for bone tissue engineering. To resolve the issue of a reduced vascular component, the reproduction of local microenvironment and the organization of cells are regarded as ultimate goals. In biofabrication, parallel to inkjet printing and extrusion-based deposition, the Laser-Assisted Bioprinting (LAB) is an alternative method for the assembly and micropatterning of biomaterials and cells. This technology allows fabrication of 2 and 3 dimensional tissue engineering constructs. The objective of this work was to promote the creation of a vascular network by different methods based on LAB, in order to optimize bone regeneration by tissue engineering.

Methods

The LAB workstation comprised a laser (λ=1064 nm, 30 ns), focused on a quartz ribbon that was coated with a thin absorbing layer of gold (60 nm) and a 30 µm layer of laser bioink. Two strategies were developed to study the creation of capillary-like structures. The first method consisted in seeding HUVECs monoculture on collagen patterns printed onto agarose. The second method consisted in a coculture obtained by printing HUVECs patterns on collagen and mesenchymal stem cells (SCAPs).

Results

HUVECs were patterned in 10mm length lines and a width between 150 and 300 microns. In monoculture, self-assembly of cells was observed in less than an hour. Capillary-like structures emerged in 48h. In coculture, vascular network was obtained in 7 days. These results imply that LAB allows printing of collagen and cells with micrometric resolution to promote *in vitro* development of vascular-like structures by organizing HUVECs.

Conclusions

This study explored two strategies to organize HUVECs by LAB. The results demonstrate that LAB is a relevant method for micropatterning HUVECs and is adapted to promote the creation of vascular-like structures in a context of bone regeneration. These capillary-like structures obtained by LAB could be included into three-dimensional constructs in order to improve angiogenesis, which is an essential prerequisite for bone healing. LAB could also allow development of vascularized bone grafts by *in situ* bioprinting of endothelial cells and osteoblast precursors. It would be a new therapeutic approach promoting bone regeneration.

Disclosure

The authors would like to thank IFRO (Institut Français pour la Recherche Odontologique) for providing financial support to this project. The authors declare that there are no conflicts of interest.

OP-049 Proposal for a new standard: quality parameters and test requirements for cell based medical products

*S. Kloth[1]

[1]OTH Regensburg, Maschinenbau, Regensburg, Germany

Objective

The standard ISO 13022 defines requirements for the risk management of cell-based medicinal products. This standard as well as several European guidelines ask for testing of the quality of raw materials, intermediates and/or final products. The publicly available specification, BSI PAS 83, lists a number of analytical tools that might be applicable for this purpose. However, a complete framework that defines specific parameters for testing of the quality of cell-based products is missing. Therefore, the German national working group M21 AK1 under the auspices of DIN is currently looking into identification of quality parameters and test requirements for cell-based products which are not covered by the established documents. Here, we present the current status of this work and ask for input of interested parties willing to take part in the process.

Method

Information was compiled from reports of national and international working groups active in the field of standardization. Members from the following groups have provided input so far: M21 Tissue Product safety (DIN), ISO TC 150 SC7 WG1: Implants for surgery / Tissue engineered medical products, ISO TC 194 SC1 WG1: Biological evaluation of medical devices, the British Standards Institution (BSI), and CEN TC 316 Medical products utilizing cells, tissues and their derivatives

Results

The document under development will focus on basic requirements for methods to be applied for quality testing of cell-based medical products. Products utilizing viable or non-viable human or animal cells will be covered. The document may be applied for products using genetically modified cells.

General requirements for test systems such as robustness, reliability, reproducibility (intralab & interlab), and precision have been established in the past. The new standard will focus on the specific characteristics of cell-based medical products.

The intended use of a product and the physical parameters of product design will establish the basis of the assessment scheme. The purpose of the assessment is to ensure patient safety by applying products of adequate quality.

As of now, two groups of tests have been defined:

- A – Methods for the testing of product characteristics
- B – Methods of testing for the potential interaction of the product with the living organism

In the first group we are suggesting the following approach for the selection of methods for the testing of product characteristics:

- Intended use: Identify the function to be replaced or added to the patient's body.
- Qualitative description of the parameters characterizing the function of the product.
- Quantitative description of the parameters characterizing the function of the product.
- Selection of suitable test method(s) for the verification of relevant qualitative and quantitative product parameters.

In the second group the following distinction might be helpful: Will the product be stably integrated in the patient's body without modification or is the product to be remodeled or completely resorbed?

Furthermore, additional aspects are to be considered:

- Biocompatibility (toxicity, pyrogens, foreign body reaction, immunogenic responses)
- Degradation products
- Biodistribution

OP-049

- - Corrosion
- - Potential for eliciting a tumor response

The members of the working group have agreed that the most challenging aspect for the selection of a relevant test battery is the high degree of variability of the biological product. In addition, lack of established reliable test methods for some valid aspects such as the tumorigenic potential of the product is another matter of concern.

OP-051 3D printing of bone/cartilage substitutes based on bioceramics/polymers

N. Sergeeva[1], *V. Komlev[2], I. Sviridova[1], V. Kirsanova[1], A. Fedotov[2], A. Teterina[2], Y. Zobkov[2], S. Barinov[2]

[1]P.A. Herzen Moscow Cancer Research Institute – National Medical Research Radiological Centre, Moscow, Russian Federation

[2]Russian Academy of Sciences, A.A. Baikov Institute of Metallurgy and Materials ScienceA.A. Baikov Institute of Metallurgy and Materials Science, Moscow, Russian Federation

Development of individual biomedical products designed to restore function and regeneration of lost tissue in the extended (by volume) bone/cartilage defects, is one of the most important and urgent tasks of the practice of dentistry, maxillofacial surgery, orthopedics, oncology and neurosurgery. The major drawback of these products is the absence of freely modality that makes possible their fabrication as patient specific implants. One of the most promising, in our opinion, solutions of this problem is the combination of advanced additive technologies, providing a layered material synthesis (mainly based on calcium phosphates and biopolymers). In this study we demonstrate that combination of three-dimensional (3D) printing with post-treatment methodology is a suitable approach to overcome current limitations in effective and fast fabrication of individual constructions for guided bone/cartilage regeneration. We proposed and developed a relatively simple route and materials for 3D printing process, targeted to production of complexly shaped and structured calcium phosphate/biopolymers grafts. The 3D printed blocks were further investigated *in vitro* and implanted in the developed in ortopic model. It was shown that these blocks were non toxic, had adhesive (for cells) properties and as a result were cytocompatible. Histological evaluation revealed that 3D printed implants were biocompatible: material directly contacted with original tissue with very thin vascularisated fibrous capsule and slight connective tissue areas making them apart.

Acknowledgments

This work was supported by the Russian Ministry of Education and Science (agreement nº. 14.604.21.0132).

OP-052 Niche-mimetic ECM scaffolds maintain hematopoietic stem and progenitor cells with a native mechanical phenotype during *in vitro* expansion

*M. Kraeter[1], A. Jacobi[2], M. Wobus[1], O. Otto[2], K. Mueller[1], J. Guck[2], C. Werner[3], M. Bornhaeuser[1]

[1]University Hospital Carl Gustav Carus, TU Dresden, Department of Medicine I, Dresden, Germany
[2]Technische Universität Dresden, Biotechnology Center, Dresden, Germany
[3]Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials, Dresden, Germany

Objective

In the bone marrow (BM) niche human hematopoietic stem and progenitor cells (hHSPCs) reside beside various cell types like mesenchymal stromal cells (MSCs) and are controlled by multiple factors such as cytokines, adhesive interactions and physical cues. Extracellular matrix (ECM) is increasingly recognized as an essential player in the regulation of stem cell homing and differentiation. To generate and characterize suitable culture methods we used decellularised ECM-scaffolds derived from BM-MSCs as substrate for hHSPC short term culture.

Material and Methods

An anchoring technic on the basis of aminosilan and polyoctadecylmethacrylate on glass coverslips was used to covalently bind fibronectin (FN) as a linker to cell secreted proteins when grown on these slips. Freshly isolated CD34+ hHSPCs were directly cultured on decellularised scaffolds and analyzed after 5d, 7d and 11d using flow and real-time deformability cytometry (RT-DC). With this technology, single cells are flowed through a microfluidic channel constriction and deformed without contact by shear stress.

Results

After seeding CD34+ cells to the ECM-scaffolds 10 to 20 % of cells adhere to the native protein environment and show clustered accumulation. This suggests that these cells retain their attachment capabilities whereas the remaining majority do not attach, which is also found in direct co-culture models of MSCs and HSPCs. ECM substrates showed 2-times stronger proliferation support for HSPCs compared to plastic culture dish (PCD)-based cultures. Beside this, CD34 positive cells expand only modestly on PCD (1,6 fold), whereas ECM scaffold cultures amplified CD34-cells up to 4 fold after 11 days. Interestingly, the proportion of attached cells does not increase after 7days and stagnated between 20 and 30 thousand cells per square centimeter ECM. After removing supernatant cells we detected proliferation of attached cells and repopulation of the supernatant.
As a label free marker for cell characteristics, we analyzed biophysical properties by measurement of short term mechanical phenotype and found first of all a rapid increase in cell size in the two populations (attached and supernatant) and PCD cultured cells after 5 days. Freshly isolated cells showed a stiff and nearly undeformable phenotype. After 5 days PCD cultured cells both adherent and in the supernatant become a heterogeneous population with stiff and soft cells. In contrast, when cells attach to ECM-scaffolds their physical properties mostly resemble that of freshly isolated CD34+ cells. Over time, the cell stiffness again increases in all populations, however, after 11 days primarily the supernatant cells start to form 2 populations which correspond to peripheral blood mononuclear cells indicating predominant differentiation behavior in this ex-vivo culture model.

Conclusion

In summary, CD34+ hHSPCs recognize decellularised ECM-scaffolds as substrates but due to their heterogeneity not all cells adhere to the provided protein structures. Compared to plastic culture dishes the matrices supported CD34+ cell proliferation significantly and lead to stiffer cell mechanical phenotype of attached cells comparable to the naïve phenotype. These data suggest that MSC-derived ECM structures may provide cytokines and biophysical signals to hHSPC which mimic aspects of the physiological niche environment during in-vitro culture.

OP-054 Effects of the scaffold architecture and implantation site on *in vivo* vascularization of polylactide-based scaffolds for potential applications in islets transplantation

n. Kasoju[1], *D. Kubies[1], E. Fábryová[2], J. Kříž[2], M. Kumorek[1], E. Sticová[3], D. Jirák[2], F. Rypáček[1]

[1]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[3]Third Faculty of Medicine, Charles University in Prague and Faculty Hospital of Kralovske Vinohrady, Prague, Czech Republic

Objective

The pancreatic islets transplantation (ITx) is an effective therapeutic alternative for type-1 diabetes mellitus. Owing to serious complications associated with the current protocol of intra-hepatic portal vein ITx, intense efforts are on to bioengineer an extra-hepatic site. But the previous reports on the biomaterials based ITx approaches showed poor or no long-term viability, majorly due to lack of vasculature that supports high oxygen demands of islets. To this end, here we aim to create a novel sub-cutaneous highly-vascularized bioartificial cavity using the polylactide-based scaffold as a template and the host body as a bioreactor. In particular, we demonstrate the effects of scaffold architecture and implantation site on the scaffold vascularization.

Materials and Methods

Polylactide-based capsular-shaped anisotropic channeled porous scaffolds were prepared using the Dip-TIPS (dipping followed by thermally induced phase separation) setup. Polyamide mesh-based isotropic regular porous scaffolds were obtained commercially and were used as the controls. The inner lumen of the scaffold was blocked by Teflon bars. They were then implanted under skin and in omentum of Brown Norway rats. The animals were examined using dynamic contrast-enhanced MRI to determine the vascular density in and around the implants. After 4-weeks, the implants were excised and analyzed by hematoxylin and eosin (HE), Masson's trichrome (TRI) and anti-CD31 antibody (CD31) staining.

Results and Discussion

The HE and TRI staining indicated a) no significant infiltration of inflammatory cells, b) minimal or no pathological fibrotic response around the scaffolds, c) guided infiltration of host connective tissue cells through the channeled pores of the test scaffold in contrast to random cell infiltration in the regular porous control scaffold, and d) the scaffolds implanted in the greater omentum appeared to have relatively superior cell infiltration than the scaffolds implanted under skin. Further, the MRI revealed that the vascular density was higher around the scaffolds implanted in omentum than the ones under skin, but, the anti-CD31 immuno-histochemistry revealed that the vascular network was present mostly on the external surface of the scaffolds.

Conclusion

Here we report a novel approach to bioengineer a sub-cutaneous bioartificial cavity using the polylactide-based scaffold as a template and the host body as a bioreactor. *In vivo* studies showed the ability of scaffold pore architecture to guide cell infiltration and the need to incorporate proangiogenic factors to enhance the vascularization in the subcutaneous space. Taken together, the current study is a significant step-forward in the field of biomaterials based extra-hepatic islet transplantation.

Supported by Ministry of Education, Youth and Sports of the Czech Republic (EE2.3.30.0029), Ministry of Health, Czech Republic (00023001, NT14240-3) and European Regional Development Fund (CZ.1.05/1.1.00/02.0109).

OP-055 *In vivo* very small embryonic-like stem cells are activated by acute tissue injury and participate in regeneration of ischemic tissue

*A. Labedz-Maslowska[1], E. Karnas[1], T. Brzozowski[2], Z. Madeja[1], E. K. Zuba-Surma[1]

[1]Jagiellonian University, Department of Cell Biology, Krakow, Poland
[2]Jagiellonian University Medical College, Department of Physiology, Krakow, Poland

Introduction

The adult murine bone marrow (BM) harbors Sca-1+/Lin-/CD45- population enriched in Oct-4+/ Nanog+ very small embryonic-like stem cells (VSELs), which may express early differentiation markers of several lineages including vascular cells. During tissue injury, BM-derived VSELs are mobilized into peripheral blood (PB). Moreover, these primitive stem cells can participate in heart repair after injection into infarcted myocardium by enhancing tissue perfusion and angiogenesis.

Question

In this study, we examined if acute tissue injury may stimulate both proliferation of quiescent VSELs in BM and their mobilization into PB *in vivo*. We also investigated genetic status of VSELs following tissue injury including expression of genes controlling their proliferation. The other goal was to evaluate regenerative potential of VSELs injected into ischemic tissues in murine model of limb ischemia (LI) *in vivo*.

Methods

C57BL/6 mice underwent LI by permanent proximal femoral artery occlusion. Mice were administrated with BrdU and scarified at 2, 7, 14 and 28 days following LI. The presence of proliferating ($BrdU^+$): VSELs ($Sca\text{-}1^+/Lin^-/CD45^-$), endothelial progenitor cells (EPCs; $Flk\text{-}1^+/Sca\text{-}1^+/Lin^-/CD45^{-/dim}$) and hematopoietic stem/progenitor cells (HSPCs; $Sca\text{-}1^+/Lin^-/CD45^+$) in PB and BM was evaluated by flow cytometry and ImageStream system. The expression of genes related to the presence of VSELs and EPCs was examined by real-time PCR. Moreover, we examined the change in secretion of angiogenesis-related proteins in plasma. Then, eGFP VSELs sorted from ischemic and non-ischemic mice were injected into site of injured tissue in WT mice at 2d following LI. At 2, 7, 14 and 28 days post transplantation, blood flow were measured by Laser Doppler System. Paraffin-embedded ischemic tissue sections were analyzed for eGFP VSELs presence and formation of new blood vessels.

Results

We found that the number of proliferating VSELs and EPCs was significantly increased in BM tissue and circulating in PB of ischemic mice at 7d post injury. Elevated number of $BrdU^+$ VSELs was accompanied with change in expression of genes guiding their proliferation as well as was correlated with blood flow recovery in both ischemic limb tissues and femoral artery indicating the role of these SC subsets in regeneration. Moreover, changes in secretion of selected angiogenesis- related proteins may stimulate activation and proliferation of VSELs. Importantly, VSELs injected into ischemic tissues enhance the improvement in tissue perfusion and blood flow recovery by formation of new blood vessels.

Conclusions

The data indicates vast impact of acute injury on activation of VSELs proliferation *in vivo* and possibility of their application for tissue regeneration.

OP-056 Healing versus regeneration process in mammals: Key role of ROS production

*E. Labit[1]

[1]STROMALab, Toulouse, France

Question

Some organisms such as salamanders have the remarkable ability to regenerate complex structures such as limbs after amputation. In mammals, this regenerative capacity is impaired by the formation of a fibrotic scar, which allows faster healing but affects the function of the repaired tissue. However, some mammals, like the MRL (Murphy Roths Large) mouse strain, display regenerative capacities. Although some reports underline the essential role of reactive oxygen species (ROS) generation peaking during the lower vertebrates' regeneration process this role has not yet been described in mammals. We aim at studying the processes involved in redirecting tissue healing towards regeneration.

Methods

We took advantage of the MRL mouse model in which we partially removed adipose tissue (AT). C57BL/6 was used as controls. AT has been chosen as a model considering that it possesses mandatory component for regeneration (immature cells, immune cells, vascularization and innervation). We investigated the repairing events after the lesion in both mouse strains, focusing on the role of ROS production in this process.

Results

We found that unlike C57BL/6 mice, MRL mice were able to regenerate AT after partial removal evidenced by a regrowth of a structurally well-organized AT. In contrast to C57BL/6 mice MRL mice presented rapid and robust inflammatory phase. Furthermore, a tissue remodeling stage was only observed in the MRL mice, characterized by the increase of MMP9 and decrease of TIMP-1, two weeks after tissue lesion. Confocal imaging then revealed that regenerated area presented very low collagen content, a large number of adipocytes clusters, vascularization and innervation. At last, we observed an early peak production of ROS, the inhibition of which prevented MRL AT regeneration.

Conclusions

For the first time, our work highlighted the central role of ROS that might reprogram mammal tissue healing towards proper regeneration.

OP-057 Silencing HLA class-I expression in allogeneic endothelial cells maintains morphological and functional properties necessary for vascular tissue engineering

*S. Lau[1,2], C. Figueiredo[3], C. Schrimpf[2], M. Pflaum[2,4], B. Wiegmann[2,4], A. Haverich[1,2,4], M. Wilhelmi[1,2], U. Böer[1,2]

[1]Hannover Medical School, GMP Model Laboratory for Tissue Engineering, Hannover, Germany
[2]Hannover Medical School, Division for Cardiothoracic-, Transplantation- and Vascular Surgery, Hannover, Germany
[3]Hannover Medical School, Institute for Transfusion Medicine, Hannover, Germany
[4]Hannover Medical School, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover, Germany

Objective

Tissue engineered vascular grafts generated from autologous tissue sources are promising alternatives to allogeneic or xenogeneic approaches. Peripheral blood is an easily accessible autologous cell source for endothelial cells which are essential for vascular grafts due to their anti-thrombogenic and vessel tone regulating properties. However, their expansion is restricted to young donors (up to 27 years) which limits their application in the clinic. Thus, allogeneic endothelial cell sources like the umbilical cord vein and umbilical cord blood have to be considered which however bear the risk of rejection after allotransplantation. Here we investigate whether silencing the human leucocyte antigen (HLA) class-I expression, which is responsible for graft rejection, affects endothelial cell morphology and function and whether HLA class-I silenced endothelial cells might be an option for tissue engineering vascular grafts. Therefore, endothelial cells from peripheral blood (PB-OEC), umbilical cord blood (UC-OEC) and the umbilical cord vein (HUVEC) were HLA class-I silenced and their morphological and functional properties were compared.

Materials and Methods

PB-OEC were derived from magnet-sorted CD34+ peripheral blood mononuclear cells (MNC) after 10-14 days of cultivation and UC-OEC were derived from umbilical cord blood MNC after 5 days of cultivation. HUVEC were purchased from Pelo Biotech. HLA class-I expression was silenced in PB-OEC, UC-OEC and HUVEC using a lentiviral vector system that delivers short hairpin RNA to silence the β-2 microglobulin domain of the HLA class-I complex. Before and after silencing endothelial cells were characterized for endothelial cell markers (CD31, CD144, vWF, eNOS) by immunostaining, their ability to form capillary-like structures in a three-dimensional fibrin gel and their orientation under laminar flow.

Results

Non-silenced PB-OEC, UC-OEC and HUVEC expressed CD31, CD144, vWF and eNOS, formed capillary-like structures in a fibrin gel and orientated themselves under laminar flow at moderate (25 dyn/cm^2) and high physiological arterial (42 dyn/cm^2) shear stress. Flow cytometric analysis of silenced endothelial cells showed a HLA class-I silencing efficiency of 85% (PB-OEC), 90% (UC-OEC) and 80% (HUVEC). Either endothelial cell type showed an unchanged expression of each characteristic endothelial cell marker, tube formation capacity and orientation under laminar flow indicating an intact functionality after silencing.

Conclusion

Allogeneic HLA class-I silenced endothelial cells have the potential to be used for the generation of pre-vascularized vascular prostheses thereby minimizing the risk of graft rejection. In particular, silenced umbilical cord blood derived endothelial cells might be of interest due to the quick and easy isolation process, the high cell yield and high proliferation capacity.

OP-058 Cellular immunotherapy using dendritic cells against multiple myeloma: from bench to clinic

*J.- J. Lee[1]

[1]Chonnam National University Hwasun Hospital & Chonnam National University, Department of Hematology-Oncology, Jeollanamdo, South Korea

Although the introduction of high-dose therapy with hematopoietic stem cell transplantation and the development of novel molecular targeting agents have resulted in a marked improvement in overall survival, multiple myeloma (MM) still remains incurable. Alternative approaches are clearly needed to prolong both disease-free and overall survival of patients with MM. Cellular immunotherapy with dendritic cells (DCs) is emerging as a useful immunotherapeutic tool to treat MM. *Ex vivo*-generated DCs can be loaded with myeloma-associated antigens as vaccines for patients with MM. The use of immature DCs or mature DCs, method used to induce DC maturation, types of tumor antigens, techniques used to load tumor antigens onto DCs, routes of administration, and dosing schedules are being investigated. Our group tried to develop the potent DCs having a capacity of high IL-12p70 production and high migratory character for inducing effective tumor-specific type I immune responses. In this presentation, I will discuss how the efficacy of DC therapy in MM can be improved. In addition, I will briefly talk to our ongoing phase I/IIa clinical trial using DCs in patients with relapsed or refractory MM.

OP-059 Chondrogenic microtissues with a lower differentiation degree used as *in vitro* transplants show improved integration in the tissue repair process in an *in vitro* therapy culture system

*M. Lehmann[1,2], F. Voß[1], F. Martin[1,2], H. Richter[3], U. Anderer[1]

[1]BTU Cottbus - Senftenberg, Cell Biology and Tissue Engineering, Senftenberg, Germany
[2]University of Leipzig, Institute of Clinical Immunology, Medical Faculty, Leipzig, Germany
[3]Clinical Centre Niederlausitz, Trauma Surgery and Orthopaedics, Senftenberg, Germany

Objectives

Cartilage defects due to traumatic injuries or also in degenerative diseases like osteoarthritis lack appropriate self-repair and require therefore cell based therapies for tissue regeneration. To avoid the application of dedifferentiated single cells as therapy basis, pre-differentiated cell aggregates may be more suitable as transplants. To test this hypothesis an appropriate *in vitro* cartilage defect environment must be provided. In this study rough-textured surfaces of articular cartilage from osteoarthritis (OA) patients after total knee joint replacement were used as cartilage defects to be repaired *in vitro*. To evaluate the *in vitro* therapy process, the integration of the transplants into the OA defects as well as repair tissue formation was monitored. Additionally, morphology and maturation of the tissues were examined.

Material and Methods

Three-dimensional cell aggregates were cultured in medium supplemented only with human serum or further differentiation promoting additives like TGF-β2 and/or L-ascorbic acid. Pre-differentiated spheroids were transferred to the naturally formed OA-defects on human condyles representing an *in vitro* therapy culture system. The tissue regeneration process *in vitro* was analysed on cryosections using histology (hematoxylin and eosin, Safranin O) as well as fluorescence-based immunohistochemistry (collagen type I and type II, S100).

Results and Conclusions

The chondrogenic microtissues integrated well into the OA-defects and started a repairing process. The level of transplant integration, cell migration into fissures and repair tissue maturation was dependent on the pre-differentiation via human serum, TGF-β2 and/or L-ascorbic acid. Interestingly, excellent integration and migration rates are contrary to well pre-differentiated *in vitro* tissues. Consequently, this therapy mimicry may be trend-setting for the development of new cell-based therapies using cartilage tissue engineering.

OP-060 Raw materials in the manufacture of advanced therapies medicinal products: quality attributes and quality assurance

*B. Leistler [1]

[1]CellGenix GmbH, Freiburg, Germany

Cytokines, growth factors and media are commonly used in the processing of cells for therapeutic applications. The quality of these raw materials (also called ancillary materials, AM) is crucial for the quality, safety and efficacy of the finished therapeutic product.

In recent years regulatory agencies recognized an increasing need for guidance for raw materials used for the production of ATMPs and started developing guidelines that outline general risk-mitigation strategies and qualification programs which can be used to select appropriate reagents (like USP General Chapter <1043> "Ancillary materials for cell, gene, and tissue-engineered products") or EP 5.2.12. "Raw materials for the production of cell-based and gene therapy medicinal products" (currently in preparation) or which outline specific quality attributes for cytokines and growth factors (like USP Chapter <92> "growth factors and cytokines used in cell therapy manufacturing").

Despite arising guidance in this area, manufacturers of AM have to identify critical quality requirements to meet increasing quality and safety concerns. Therefore, the origin, composition, manufacturing process, QC methods and release specifications of AM have to be shared between AM manufacturers, regulatory agencies and ATMP manufacturers. The use of GMP grade and animal-derived component-free (ADCF) manufactured ancillary materials, derived from well-characterized cell banks, will significantly reduce qualification and validation efforts of cell therapy manufacturers and help to ensure consistency, safety and purity of the final cell therapy products.

OP-062 From hair to repair: potentials of MSCORS in chondrogenesis and cartilage repair

*H. Li[1], S.- J. Yun[2], J.- K. Seon[2], J.- C. Simon[1,3], V. Savkovic[1]

[1]Translational Centre for Regenerative Medicine (TRM) Leipzig, CELLT, Leipzig, Germany
[2]Chonnam National University Hwasun Hospital , Gwangju, South Korea
[3]Faculty of Medicine, Universität Leipzig, Clinic for Dermatology, Venerology and Allergology, Leipzig, Germany

Outer Root Sheath (ORS) of human hair follicle harbors variety of stem cells and precursors that are inherently responsible for hair cycling and skin regeneration. This small compartment provides infinite possibility to yielding somatic stem cells and developing tissue engineering treatments for Regenerative Medicine. In past few years, we developed a non-invasive autologous cell therapy using plucked hair follicles to isolate Mesenchymal Stem Cells from Outer Root Sheath (hf-MSCORS), and identified therapeutic chondrogenic potentials of MSCORS, further developing chondrogenic differentiation for unmet clinical needs of treating articular cartilage deficiencies with autologous, non-invasively sampled, differentiated cells.

MSCORS are released from the ORS of plucked hair follicles, expanded *in vitro* and characterized with human MSC markers (line CD44+, CD90+, CD105+, CD146+, CD166+, Stro-1+, CD19- and CD45-, CD34-). MSCORS exhibit MSC-like properties and express typical MSC markers in gene and protein level in accordance to the ISCT criteria. Through immunofluorescent staining and FACS, we identified location and population of MSCORS in the ORS, and discovered that MSCORS from plucked follicles possess high viability and an outstanding marker expression. MSCORS showed good multipotency in the MSC differentiation assay. By employing 3D dimensional cells culture and human adipose-derived MSC, we have successfully established a standardized procedure for chondrogenic differentiation and constructs with promising chondrogenic gene expressions and cartilaginous ECM accumulation, confirming the functional chondrogenesis.

Future work requires standardizing the MSCORS scale-up with phenotypic maintenance and full characterizations. 3D chondrogenesis and tissue engineering needs to be translated from mouse models to human MSCORS. With full therapeutic potentials and efficiency of MSCORS, they carry a great potential for an Advanced Therapy Medicinal Product as functional Superficial Articular Cartilage that should be developed towards the clinical applications on Osteoarthritis treatments and cartilage repair.

OP-063 Human heart valve-derived scaffold improves cardiac repair in a murine model of myocardial infarction

L. Wan[1], Y. Chen[1], Z. Wang[1], W. Zhang[1], S. Schmull [1], W. Gao[1], S. Xue[2], J. Dong [3], H. Imboden[4], *J. Li[1]

[1]Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Renji-Med X Clinical Stem Cell Research Center, Shanghai, Germany
[2]Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Cardiovascular Surgery, Shanghai, China
[3]German Rheumatism Research Centre, Berlin, Germany
[4]Institute of Cell Biology, University of Bern, Bern, Switzerland

Cardiac tissue engineering using biomaterials with or without a combination of cardiac stem cell therapy offers a new therapeutic option for repairing infarcted heart. So far, cardiac tissue scaffold is designed mainly based on natural and synthetic biomaterials, which do not mimic the biochemical components and structural properties of native human myocardial extracellular matrix. This raises a fundamental issue about the biocompatibility of currently used biomaterials with myocardial tissue. Here we hypothesized that the human heart valve-derived scaffold (hHVS) may provide a clinically relevant novel biomaterial for cardiac repair. In this study, human heart valve tissue was sliced into 100 μm tissue section by frozen-sectioning and then decellularized to form the hHVS. Upon anchoring onto the hHVS, post-infarct murine BM c-kit+ cells exhibited an increased capacity for proliferation and cardiomyogenic differentiation *in vitro*. When used to patch infarcted heart in a murine model of myocardial infarction, either implantation of the hHVS alone or c-kit+ cell-seeded hHVS significantly improved cardiac function, as shown by transthoracic echocardiography as well as by hemodynamic measurements via a Millar catheter, and by reduced infarct size; while c-kit+ cell-seeded hHVS was even superior to the hHVS alone. Thus, we have successfully developed a hHVS for cardiac repair. Our *in vitro* and *in vivo* observations provide the first experimental evidence for translating the hHVS-based cardiac tissue engineering into clinical strategies to treat myocardial infarction.

OP-064 Biogelx Ltd: designer gels for cell culture

*D. Lightbody[1], E. Irvine[1]

[1]Biogelx Ltd, Lanarkshire, Great Britain

Biogelx Limited is a biomaterials company that designs tuneable peptide hydrogels, offering artificial tissue environments to cell biologists for a range of cell culture applications.

The hydrogels are highly tuneable, cell-matched biomaterials, capable of revolutionising the way cell biologists control and manipulate cell behaviour in the laboratory. This is of direct relevance to fundamental cell research, including the study of stem cells and disease models within academic and medical labs. However, the major commercial significance this has is the dramatic impact on the development of cell-based assays and drug discovery/toxicology platforms within large pharmaceutical companies, representing a rapidly growing global market with revenues of over $5 billion per annum.

Biogelx offers a range of hydrogel platforms that are three dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue (see figure below). This gives control back to the cell biologist, as the gels can be tuned to meet the needs of any given cell type.

This presentation will discuss the underlying chemistry of Biogelx's peptide hydrogels, highlighting the range of chemical and mechanical modifications that can be implemented within the gels, in order to address a wide range of cell based applications. Some examples of academic and industrial collaborative work shall also be presented. Finally, the company's progress from research-based technology through to commercial entry into high value markets will be discussed, highlighting Biogelx's current successes and future aspirations.

OP-066 High density pellet culture for human knee meniscus tissue formation using a unique combination of medium

Mamatha M. Pillai[1], V. Elakkiya[1], J. Gopinathan[1], C. Sabarinath[2], K. Santosh Sahanand[3], B. K. Dinakar Rai[4], Amitava Bhattacharyya[1], R. Selvakumar[1,*]

[1]PSG Institute of Advanced Studies, Tissue Engineering, Coimbatore, India
[2]PSG College of Technology, Biotechnology, Coimbatore, India
[3]Ortho one orthopedic specialty centre , Coimbatore, India
[4]PSG Institute of Medical Sciences and Research, Orthopeadics, Coimbatore, India

Aim
The present study was carried out to investigate the impact of unique combination of biomolecules like chondroitin sulphate, proline, biotin and glucose on stimulation of meniscus cell growth, proliferation and tissue formation using high density pellet culture.

Methods
The isolated human knee meniscus cells were treated with above mentioned biomolecules as individual and in combination to study possible stimulation of cells growth and proliferation. The cell proliferation was found to vary from one biomolecule to another and with its combination. By using this unique combination of biomolecules, time required to reach 100% confluency in a 60 mm plate was reduced from 36 days to 4 days as compared to control. The growth rate of human meniscus cells were studied through analysis of doubling time, histology, viability tests and gene expression analysis. Meniscus cells expanded *in vitro* were cultured to form micro-sediments and treated with unique combination of medium. Tissue was formed within 30 days and were fixed and prepared for histological and Immunohistochemical analysis. Sections were stained with Sirius red for collagen detection and quantitative estimation of extracellular matric (ECM) production was performed. Transmission electron microscopy (TEM) was used to observe the ultrastructure of ECM in 3D cultures.

Results and conclusion
In this 3D culture system, instead of fibroblast-like morphology seen in usual monolayer culture cells morphology was switched to spherical shape. This spherical morphology of typical human meniscus cells was achieved in 3D pellet culture by using supplementation of unique combination of biomolecules. Besides that, cells synthesized ECM and showed high production of glycosaminoglycans and collagen compared to control. This 3D culture system is effective for monitoring cartilage formation better than other methods described in the literature and could serve as an excellent model for cartilage tissue engineering.

OP-067 Cell sheet engineering: approach for treatment of ischemic disorders via delivery of VEGF165-expressing ADSC

*P. Makarevich[1,2], K. Dergilev[2], E. Gluhanyuk[1], Z. Tsokolaeva[2], A. Efimenko[1], P. Rodina[1], J. Gallinger[1], S. Sarkisyan[1], Y. Parfyonova[2], Y.- C. Hu[3]

[1]Lomonosov Moscow State University, Faculty of Medicine, Moscow, Russian Federation
[2]Russian Cardiology Research and Production Complex, Laboratory of Angiogenesis, Moscow, Russian Federation
[3]National Tsing-Hua University, Department of Chemical Engineering, Hsinchu, Taiwan, Province Of China

Question

Delivery of cells for therapeutic application is a well-established approach to trear limb ischemia and a range spectrum of other disorders. Still efficacy of cell therapy is hindered in a number of trials due to a number of factprs including low survival of transplanted cells. This obstacle can be circumvented by application of tissue-engieered constructs, e.g. cell sheets (CS), which comprise of cellular elements along with their extracellular matrix.

Methods

We established and effective and rapid protocol for generation of CS from adipose-derived stromal cells (ADSC) of human and animal origin without appliaction of thermoresponsive dishes. Detached CS were transplanted to mice with induced limb ischemia (n=8-10 per group) by subcutaneous attachment after placement of exposed skeletal muscle. In some animals CS were transduced using a hybrid baculoviral system to express VEGF165, which enahnces ADSC therapeutic potential. Suspended ADSC were also deliverd via injections to serve as reference method. Limb perfusion was assessed via laser Doppler and histology inclided routine stains and analysis vascular density (stains for CD3 and a-SMA), proliefration (Ki-67) and apoptosis (caspase-3) in muscle and CS.

Results

In all specimen analzyed after animal euthanasia we found CS to be engrafted, vascularized and infiltarted by CD68+ monocytes at 7 and 14 days post delivery. Vacularization of CS comprised of CD31+ capillaries and a-SMA+ arterioles indicating graft-host interactions. Approximately 10% of cells within CS were caspase-3 positive indicating apopotosis and a number of Ki-67+ nuclei was found reflecting proflierating cells. These changes were accompanied by significant improvement of perfusion, vascular density and necrosis reduction indicating ischemia relief comapred to untreated control. In our study CS delivery was superior to injection of suspended ADSC in equivalent amount. In these animals we found significant engraftment of ADSC, which can be described as intramuscular infiltrates yet perfusion was lower, than in CS group. Still attempting to further enhance method's efficacy we expressed VEGF165 is CS prior to delivery and found that VEGF165-expressing CS had even stronger impact of limb perfusion reaching up to 65% per cent vs. 50-55% in unmodified CS and approximately 40% in susp. Moreover we found VEGF165-expressing CS to induce angiogenesis in a more efficient way and further stimulate capillarogenesis.

Conclusions

Overall, our data indicates that delivery of CS from ADSC is an effective way for tissue protection and stimulation of angiogenesi in ischemic limb. Delivery of CS was superior to suspension in terms of limb perfusion and angiogenesis stimulation. Still viral expression of VEGF165 has enhanced therapeitic potential of CS and allowed to robustly restore limb perfusion and reduce necrotic change to minimal extent.

OP-068 An osteogenic differentiation model of fibroblast-like synoviocytes: is there a role for pulsed electromagnetic fields?

*F. F. Masieri[1], A. Ghisellini[2], A. Rajic[1], S. Setti[3], R. Cadossi[3]

[1]University Campus Suffolk, Science and Technology, Ipswich, Great Britain
[2]University of Ferrara, Ferrara, Italy
[3]IGEA S.p.A., Laboratory of Clinical Biophysics, Carpi (Modena), Italy

Objectives

Fibroblast-Like Synoviocytes (FLSs) reside in the synovium, where they play a key role in arthritic joint diseases. Despite their mesenchymal origin, FLS tri-lineage differentiation capacity *in vitro,* particularly toward the osteogenic lineage, is still debated. This hinders their application in regenerative medicine. Pulsed Electromagnetic Fields (PEMFs) have been shown to help differentiation processes in several mesenchymal stem cell models and to counteract inflammatory pathways in FLSs *in vitro.* To this purpose, we investigated the role of selected PEMFs in supporting the osteogenic differentiation of an *in vitro* model of FLSs.

Material and Methods

SW982 cells (human synovial sarcoma cell line) were cultured for 1, 14 and 21 days in osteogenic medium, with and without PEMF stimulation. The PEMF exposure system consisted of a pair of parallel vertical Helmoltz copper coils placed inside the incubator, equidistant from the cells and generating a signal with the following characteristics: frequency 75Hz, intensity 1.5mT, pulse duration 1.3msec. Osteogenic differentiation was assessed qualitatively and quantitatively by Alizarin Red staining and Alkaline Phosphatase (ALP) activity. Furthermore, type I Collagen, matrix extracellular phosphoglycoprotein (MEPE) and runt-related transcription factor 2 (Runx-2) were analysed by immunofluorescence. Viability and metabolic activity were assessed via MTT test.

Results

After 21 days, cells exposed to osteogenic medium and PEMFs showed a significant increase in ALP activity, compared to cells grown in osteogenic medium alone and controls (+85% and +270%, respectively, $p<0.05$). This was supported by consistent ALP cytochemical staining. Mineralised matrix deposition was also observed in a time-dependent manner, in comparison to controls. At day 21, PEMFs induced a marginal but significant increase in mineralised matrix deposition, compared to cells grown in osteogenic medium alone (+13%, $p<0.05$). MEPE and Runx-2 were detected with variable intensities of staining, particularly at day 21 of differentiation under osteogenic conditions, with and without PEMFs. Interestingly, areas of organised type I Collagen fibrils were visible only in cells treated under osteogenic conditions, especially in the presence of PEMF. Cell viability was significantly increased in osteogenic medium, with and without PEMFs (+7.4folds, $p<0.05$), as detected by MTT. The higher metabolic activity may indeed be associated to the cell differentiation events observed.

Conclusions

The findings suggest that SW982 can effectively be differentiated toward the osteogenic lineage, representing a good model of FLS differentiation. PEMF stimulation seems to play an important role in further enhancing osteogenic differentiation of SW982. Taken together these results may open up new approaches for the application of specific biophysical stimulation in processes of osteogenic differentiation and ultimately in bone regenerative medicine.

OP-069 Autotransplantation of the adipose tissue-derived stem cells for venous stasis ulcers healing

M. Paprocka[1], D. Duś[1], J. Kubiak[2], E. Wojtowicz-Prus[2], A. Czarnecka[2], W. Witkiewicz[2], *L. Masłowski[2], R. Grendzial[2], A. Czyżewska-Buczyńska[2]

[1]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of Polish Academy of Science, Wrocław, Poland
[2]Regional Specialist Hospital, Research and Development Centre, Wroclaw, Poland

Objective

Adipose tissue is a reliable source of multipotent adult stem cells for use in regenerative medicine, owing to their easy accessibility with abundant numbers and association with minimal patient discomfort during procedure.

The aim of the study was the assessment of safety and efficacy of autologous adipose tissue-derived stem cells transplantation in the treatment of chronic venous stasis ulcers.

Material and Methods

Adipose tissue was harvested by aspiration after infiltration by local anesthetic and tumescend isotonic solution. Stem cells were separated using closed and fully automated adipose-derived stem cell isolation system (CELLUTION 800, Cytori Therapeutics, USA). Cell suspension concentrate (5 ml) was implanted to subcutaneous tissue around the wound and the wound bed. The phenotype of the cells obtained was evaluated by flow cytometry analysis (FACS, Becton Dickinson, USA) immediately after separation and after 7 days of culture at 37˚C/5% CO_2 in culture medium (RPMI 1640 (Gibco BRL, UK), 10% fetal bovine serum (FBS, Sigma, USA), 100 U/mL of penicillin and 100 mg/mL of streptomycin (Sigma, USA)). Specific antibodies were used as followed: anti-CD45, anti-CD31, anti-CD34, anti-CD133, anti-CD146, anti-CD105, anti-CD90 (all from BD Bioscience, USA).

Study group consisted of 14 patients (11 women and 3 men; mean age 66,6 +/- 9,5 years) with chronic venous stasis ulcers. All ulcers were assessed planimetric using VISITRAK Digital (Smith & Nephew, UK) and documented by digital photos before autotransplantation and every 2 weeks during the 6-month follow-up. After autotransplantation all patients were 6 months of follow-up and received standard local and general treatment.

Results

The preparation obtained from the separator contained an average of 5,6 x 10^6 +/- 4 x 10^6 cells per milliliter of cell suspension concentrate. The phenotype of the cells was determined after separation (CD45 23,5% +/- 2,38; CD31 20,75% +/- 5,12; CD34 71,75% +/- 19,97; CD133 0,0%; CD146 49% +/- 25,25; CD105 41,5% +/- 29,03; CD90 79% +/- 17,06) and after 7 days of culture in standard conditions (CD45 10% +/- 10,98; CD31 8% +/- 11,31; CD34 33,75% +/- 34,97; CD133 0,0%; CD146 15,75% +/- 13,37; CD105 82,25% +/- 32,84; CD90 94,5% +/- 7,05).

A common side effect of lipoaspiration was subcutaneous abdominal wall hematoma which did not required specific treatment. Other side effects were not observed. Clinical improvement was observed in 71,4% of patients: 3 patients were completely healed and 7 with >50% reduction of ulcer area. Ulcer progression was observed in 1 patient, in 3 patients - no change of ulcers size was observed. In patients responding to the regenerative therapy healing acceleration were observed to 10-12 weeks after autotransplantation.

Conclusion

Autotransplantation of adipose tissue stem cells is a save and promising treatment method for chronic venous ulcers. However, transient therapeutic effect after the single autotransplantation forces/is a contribution to the search for methods of cell propagation *in vitro* in order to repeat therapeutic procedure.

Acknowledgement

This study is a part of project „Wrovasc - Integrated Cardiovascular Centre", co-financed by the European Regional Development Fund, within Innovative Economy Operational Program, 2007-2013. European Funds - for the development of innovative economy.

OP-070 Safety study of intravenously administered human cord blood stem cells in the treatment of symptoms related to chronic inflammation

*B. Mehling[1], L. Quartararo[1], M. Manvelyan[1], P. Wang[1], D.- C. Wu[1,2]

[1]Mehling Orthopedics, Hackensack, China
[2]Biochemistry Institute, Wuhan University, Hubei, China

Background
Numerous investigations suggest that Mesenchymal Stem Cells (MSCs) in general represent a valuable tool for therapy of symptoms related to chronic inflammatory diseases. Blue Horizon Stem Cell Therapy Program is a leading provider of adult and children's stem cell therapies. Uniquely we have safely and efficiently treated over 600 patients with documenting each procedure.

Methods
The purpose of our study is primarily to monitor the immune response in order to validate the safety of intravenous infusion of human umbilical cord blood derived MSCs (UC-MSCs), and secondly, to evaluate effects on biomarkers associated with chronic inflammation. 20 patients were treated for conditions associated with chronic inflammation and for the purpose of anti-aging. They have been given one intravenous infusion of UC-MSCs.

Results
Our study of blood test markers of 20 patients with chronic inflammation before and within three months after MSCs treatment demonstrates that there is no significant changes and MSCs treatment was safe for the patients. Analysis of different indicators of chronic inflammation and aging included in initial, 24-hours, two weeks and three months protocols showed that stem cell treatment was safe for the patients; there were no adverse reactions. Moreover data from follow up protocols demonstrates significant improvement in energy level, hair, nails growth and skin conditions.

Conclusion
Intravenously administered UC-MSCs were safe and effective in the improvement of symptoms related to chronic inflammation. Further close monitoring and inclusion of more patients are necessary to fully characterize the advantages of UC-MSCs application in treatment of symptoms related to chronic inflammation.

OP-072 FLIM imaging of the mesenchymal stem cell metabolic status during differentiation

*A. Meleshina[1,2], V. Dudenkova[1,2], A. Bystrova[1,2], E. Cherkasova[1,2], E. Zagaynova[1,2,3]

[1]Nizhny Novgorod State University, Nizhny Novgorod, Russian Federation
[2]Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russian Federation
[3],, Russian Federation

Objectives

The non-invasive imaging of cell metabolism within tissues to assess the efficacy of stem cell therapy and understanding the tissue development is of great interest.

In this study we investigated metabolic trajectory of the mesenchymal stem cell (MSC) differentiation on the change of optical redox ratio of (NADH+FAD) / FAD and the fluorescence lifetime of free and bound forms of the reduced nicotinamide adenine dinucleotide (NADH) and oxidized flavin adenine dinucleotides (FAD) using Fluorescence Lifetime Microscopy.

Material and methods

Undifferentiated, adipogenically and osteogenically differentiated human MSCs were imaged with a Zeiss 710 microscope coupled to a FLIM system. The intrinsic fluorescence of NADH and FAD was excited at 750 nm and 900 nm respectively by a femtosecond Ti:sapphire laser. The data were analyzed with the commercially available SPCImage software. The result was fitted to the actual data to derive FLIM parameters (short lifetime t1, long lifetime t2, short lifetime contribution a1 and long lifetime contribution a2).

Results and conclusions

The FLIM parameters of undifferentiated, adipogenically and osteogenically differentiated MSCs were obtained. The changes in the NADH and FAD fluorescence lifetime from the undifferentiated MSCs to the differentiated adipocytes and osteoblasts were confirmed in a series of samples. Short and long NADH, FAD lifetime fluorescence were increased in the differentiated adipocytes and osteoblasts. The dynamic varying of a1/a2 ratio indicates the change ratio of free and bound NADH and FAD in differentiated MSCs. Besides the decrease of redox ratio of (NADH+FAD) / FAD during differentiation was shown. This is the evidence of changes in the ways of the hydrolytic cleavage of macromolecules in the cells.

The results suggest that the change in the redox ratio and in the ratio of free and bound forms of NADH and FAD in undifferentiated and differentiated MSCs indicates a switching pathway from glycolysis to oxidative phosphorylation in the differentiation process.

OP-074 Effect of apatite-wollastonite glass ceramics of the growth, osteogenic differentiation and migration of mesenchymal stem cells

*S. Muller[1], L. Nicholson[1], J. De Havilland[2], K. Dalgarno[3], A. Dickinson[1], X.- N. Wang[1]

[1]Newcastle University, Institute of Cellular Medicine, Newcastle-upon-tyne, Great Britain
[2]Newcastle University, Centre for Life, Newcastle-upon-tyne, Great Britain
[3]Newcastle University, Mechanical Engineering , Newcastle-upon-tyne, Great Britain

Apatite-wollastonite glass ceramic (A-W) is a biocompatible and osteoconductive material which can be easily 3D printed into a scaffold that fits the needs of a patient with a bone defect. Cells in the surrounding areas may be able to migrate towards the implanted scaffold to aid the regeneration of tissue. Alternatively, scaffolds can be seeded with osteogenic cells such as mesenchymal stem cells (MSCs) to increase the level of bone regeneration. MSCs isolated via plastic adherence are a highly heterogeneous population; however the enrichment of the sample for a specific marker could generate a population with greater osteogenic potential. CD271+ MSCs, thought to be progenitor MSCs, have been shown to be osteogenicly primed and hence may be better suited for bone regeneration.

This study compares the growth and differentiation of paired plastic adherent (PA-MSCs) and CD271 enriched MSCs (CD271-MSCs) seeded onto A-W scaffolds. Cell growth on the scaffold was measured via DNA quantification and osteogenic differentiation was assessed using qRT-PCR and microscopy. A chemotaxis assay was used to assess MSC migration towards the scaffold.

In paired samples, the CD271-MSCs seeded onto A-W scaffolds initially yielded much smaller amounts of DNA with an average 36.3 ng/ml at day 1 (±1.2 SEM) and 13.5ng/ml at day 3 (±0.7 SEM), while PA-MSCs yielded an average 58.9 ng/ml of DNA at day 1 (±1.5 SEM) and 36.9ng/ml DNA at day 3 (±0.7 SEM). After 7 days of culture on the scaffolds the DNA yield from CD271-MSC was 43ng/ml (±3.1 SEM) and only 24.4ng/ml (±1.0 SEM) from the PA-MSCs. SEM imaging at day 14 of culture confirmed that the CD271-MSCs have a greater number of cells covering the scaffolds compared to the PA-MSCs.

Despite the increased cell growth of CD271-MSCs on the scaffold RT-PCR results showed no significant difference in the expression of bone-specific RNAs between CD271-MSCs and PA-MSCs grown on A-W scaffolds.

Chemotaxis assays found that CD271-MSCs had a slower rate of migration compared to the PA-MSCs, resulting in the CD271-MSCs travelling the overall shortest distance (CD271-MSC average total distance of 2.53μm (±0.27 SEM), PA-MSC average total migration distance of 3.79μm (±0.54 SEM)).

The distance which the cells moved towards CXCL12 was similar in the two population, with CD271-MSCs moving 0.66μm (±0.16 SEM) away from their point of origin towards the chemoattractant and the PA-MSCs moving 0.61μm (±0.46 SEM) away from their point of origin towards the chemoattractant. This insinuates that, though the CD271-MSC population travelled less total distance than the PA-MSCs, their migration was more directed in the direction of the CXCL12 with less random movement.

The scaffold conditioned media had similar chemoattractant effect as media alone. Further analysis found that PA-MSC conditioned media is a strong chemoattractant whilst MSC seeded-scaffold conditioned media had minimal effects on MSC migration. Scaffold conditioned media also inhibited the chemoattractant effect of MSC conditioned media as well as CXCL12.

These results show that A-W scaffolds do not attract MSC migration. Also the scaffold conditioned media inhibited MSC migration towards known chemoattractants through an unknown mechanism. As CD271-MSCs grew better on the surface of the scaffold, further work will be done to investigate how we can enhance their osteogenic differentiation on the A-W scaffold.

OP-076 A Phase II clinical study of genetically modified cell therapy for degenerative osteoarthritis of the knee

*M. J. Noh[1], R. O. Copeland[1], H. J. Yoon[1], K. H. Lee[1]

[1]TissueGene, Inc., Rockville, United States

Question

Osteoarthritis is the most frequently encountered orthopedic disease associated with pain, inflammation and cartilage damage, and current methods for treating osteoarthritis include pharmacological treatments, physical therapy and surgery. However, these treatments do not result in physiological or structural regeneration in the damaged joint.

TG-C represents a novel technique of cell-mediated cytokine gene therapy for osteoarthritis treatment using localized delivery of allogeneic chondrocytes expressing TGF-β1 incorporated with untransduced human chondrocytes at a 1:3 ratio. A Phase II clinical trial was performed to evaluate the safety and efficacy of TG-C in patients with advanced osteoarthritis of the knee joint. Following a single intraarticular injection into the joint space of the damaged knee, patients were monitored for safety, and an evaluation was performed to assess the efficacy of TG-C.

Methods

A multi-center, double-blind, placebo-controlled, randomized study of adults with Kellgren-Lawrence grade 3 knee osteoarthritis. A total of 102 patients were randomized 2:1 to receive TG-C (n=67) or placebo (normal saline; n=35). After a single intra-articular administration of TG-C or placebo, the enrolled patients wereassessed for: (1) efficacy with regard to pain score (VAS) and knee functionality and symptoms score (IKDC) of knee osteoarthritisfor 24 months; (2) cartilage regeneration using 3.0 Tesla MRI; and (3) safety of administration and the incidence adverse events.

Results

The TG-C cohort consisted of 67 patients (24 men and 43 women) who had a mean age of 57 years (34 to 70 years), and body mass index (BMI) of 30 kg/m^2 (19 to 43). The placebo cohort consisted of 35 patients (14 men and 21 women) who had a mean age of 56 years (25 to 70 years) and BMI of 30 kg/m^2 (20 to 43).

A statistically significant improvement was observed for both efficacy parameters (IKDC and VAS) in the change from baseline for the TG-C group over the placebo group upon primary analysis of the data. For the IKDC subjective knee evaluation the change from baseline improved 13.4 points (LSMD [95% CI]: 3.3 to 23.6; p = 0.01) in the TG-C group compared to the placebo group at month 12, and 14.2 points (LSMD [95% CI]: 3.8 to 24.6; p = 0.0076) at month 24. The change from baseline in VAS pain score improved -15.5 points (LSMD [95% CI: -27.5 to -3.4; p = 0.012] in the TG-C group compared to the placebo group at month 12, and -11.1 points (LSMD [95% CI]: -23.3 to 1.1; p = 0.075) at month 24.

The MRI analysis showed cartilage regeneration evidence in some TG-C treated patients. The most common AEs definitely related to treatment with TG-C were joint inflammation, arthralgia and joint effusion. Four SAEs were experienced, two in the TG-C group and two in the placebo group. These SAEs were not considered to be related to the study medication.

Conclusion

A single intraarticular injection with TG-C was shown to be safe and demonstrated positive effects in treating OA of the knee, as evidenced by improvement in both pain and symptoms of OA. Patients receiving TG-C exhibited statistically significant improvement in responses on the IKDC subjective knee evaluation,and inpain as rated by VAS as compared to placebo. The symptomatic improvement was maintained at least 24 months after treatment. Improvements in knee articular cartilage seen on MRI may translate into improvements in pain and functional outcomes. We believe that TG-C provides a promising future treatment for osteoarthritis. These Phase II results form the basis for a planned Phase III development program that will be underway soon.

OP-076

Table 1. Summary Statistics of Visual Analog Scale for Pain

	TG-C			Placebo					
Visit	**n**	**LS Mean**	**95% CI of LS Mean**	**n**	**LS Mean**	**95% CI of LS Mean**	**LS Mean Difference**	**95% CI of LS Mean Difference**	**p-value**
Month 1	65	-26.9	(-33.4 - -20.4)	35	-19.7	(-28.5 - -10.9)	-7.2	(-18.1 - 3.8)	0.1971
Month 3	58	-37.1	(-43.8 - -30.4)	33	-22.7	(-31.6 - -13.7)	-14.4	(-25.7 - -3.2)	0.0119
Month 6	58	-35.5	(-42.2 - -28.8)	31	-27.0	(-36.1 - -17.9)	-8.5	(-19.8 - 2.9)	0.1423
Month 12	49	-39.7	(-46.7 - -32.7)	24	-24.3	(-34.1 - -14.5)	-15.5	(-27.5 - -3.4)	0.0120
Month 18	48	-39.7	(-46.7 - -32.6)	23	-23.0	(-32.9 - -13.1)	-16.6	(-28.8 - -4.5)	0.0074
Month 24	45	-34.4	(-41.5 - -27.2)	23	-23.3	(-33.2 - -13.4)	-11.1	(-23.3 - 1.1)	0.0750
Overall	65	-35.5	(-41.0 - -30.0)	35	-23.3	(-30.8 - -15.8)	-12.2	(-21.5 - -2.9)	0.0106

Table 2. Summary Statistics of IKDC Subjective Knee Evaluation for Function

	TG-C			Placebo					
Visit	**n**	**LS Mean**	**95% CI of LS Mean**	**n**	**LS Mean**	**95% CI of LS Mean**	**LS Mean Difference**	**95% CI of LS Mean Difference**	**p-value**
Month 1	47	13.6	(7.9 - 19.2)	27	11.2	(3.7 - 18.7)	2.3	(-7.1 - 11.7)	0.6249
Month 3	42	22.9	(17.2 - 28.7)	25	12.6	(5.0 - 20.2)	10.4	(0.8 - 19.9)	0.0337
Month 6	43	20.3	(14.5 - 26.1)	24	12.1	(4.4 - 19.9)	8.2	(-1.5 - 17.8)	0.0969
Month 12	38	23.3	(17.3 - 29.3)	20	9.9	(1.7 - 18.1)	13.4	(3.3 - 23.6)	0.0100
Month 18	36	20.6	(14.5 - 26.8)	18	4.8	(-3.7 - 13.2)	15.9	(5.4 - 26.3)	0.0031
Month 24	35	22.0	(15.8 - 28.2)	19	7.8	(-0.5 - 16.1)	14.2	(3.8 - 24.6)	0.0076
Overall	47	20.5	(15.6 - 25.3)	27	9.7	(3.3 - 16.2)	10.7	(2.6 - 18.8)	0.0101

OP-077 Towards modeling limb development: high-throughput microfluidic platform for culturing mesenchymal stromal cell perfused micromasses

*P. Occhetta[1,2], M. Centola[1], A. Barbero[1], B. Tonnarelli[1], A. Redaelli[2], I. Martin[1], M. Rasponi[2]

[1]University Hospital of basel, Departments of Surgery and of Biomedicine, Basel, Switzerland
[2]Politecnico di Milano, Department of Electronics, Information and Bioengineering, Milano, Italy

Question

The first stages of embryonic limb development - namely cell condensation, undifferentiated proliferation and chondrogenesis - are tightly regulated by the interplay of specific signaling pathways (Wnt, FGF, TGFβ/BMP). The development of reliable *in vitro* models to screen the effect of such morpho-regulatory factors on stem/progenitor cells is crucial to elucidate and possibly recapitulate developmental processes.

Methods

Towards this aim, we report an innovative microfluidic platform designed to generate and culture 3D stem/progenitor cells as perfused micromasses (PMMs), consisting of two functional elements: (i) a serial dilution generator (SDG) -either linear or logarithmic in shape- and (ii) a 3D culture region. Patterns of different combinations/concentrations of morphogens are generated through the SDG and delivered to downstream culture units, each comprised of 10 cubic microchambers (side 150µm).

Results

Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) cultured within microchambers were able to undergo spontaneous condensation within 3 hours upon seeding, generating PMMs uniform in size (77±15cells, Φ=56.2±3.9µm). As compared to traditional static macropellet cultures, exposure to morphogens involved in the first stages of embryonic limb development (i.e., Wnt, FGF and TGFβ pathways) yielded more uniform and repeatable responses of 3D PMMs, and a 34-fold higher percentage of proliferating cells at day7. The use of the logarithmic serial dilution generator unit then allowed screening the concentration-dependent effect of a key morphoregulatory factor (i.e. TGFβ3) on 3D hBM-MSCs PMMs, spanning over four orders of magnitude. Interestingly, the lowest concentration tested (100 pg/ml) was sufficient to trigger specific cellular responses (i.e. chondrogenic specification and cellular proliferation), which cannot be detected either at the macroscale or in 2D models, without delivering at least hundred times higher concentrations of the morphogen.

Conclusions

Allowing the investigation of developmental signals involved in the first stages of limb bud formation in a high-throughput fashion, this microfluidic platform represents a powerful tool towards the definition of 'developmental-inspired' approaches for skeletal tissue regeneration. Moreover, we are currently assessing the portability of the presented technology to different cell sources (e.g. mesenchymal limb progenitors), envisioning the possibility to extend the applicability of this platform to different biological models, where initial cell condensation and onset of a 3D structure are expected to mediate a physiological response to exogenous signals. The proposed microfluidic platform may thus find further applications in the field of developmental biology - e.g. as an *in vitro* limb bud model - as well as in the pharmaceutical industry for screening compounds intended to induce regeneration of mesenchymal tissues.

References

1 ten Berge D, Brugmann SA, Helms JA, Nusse R. Wnt and FGF signals interact to coordinate growth with cell fate specification during limb development. *Development* 135, 3247–3257 (2008).

2 Centola M, Tonnarelli B, Schären S, Glaser N, Barbero A, Martin I. Priming 3D cultures of human mesenchymal stromal cells toward cartilage formation via developmental pathways. *Stem Cells Dev.* 22, 2849–2858, (2013).

Disclosure

This work was partially funded by the Swiss National Science Foundation program, SNF grant no. NBM 1579, and by Fondazione Cariplo, grant no. 2012-0891.

OP-077

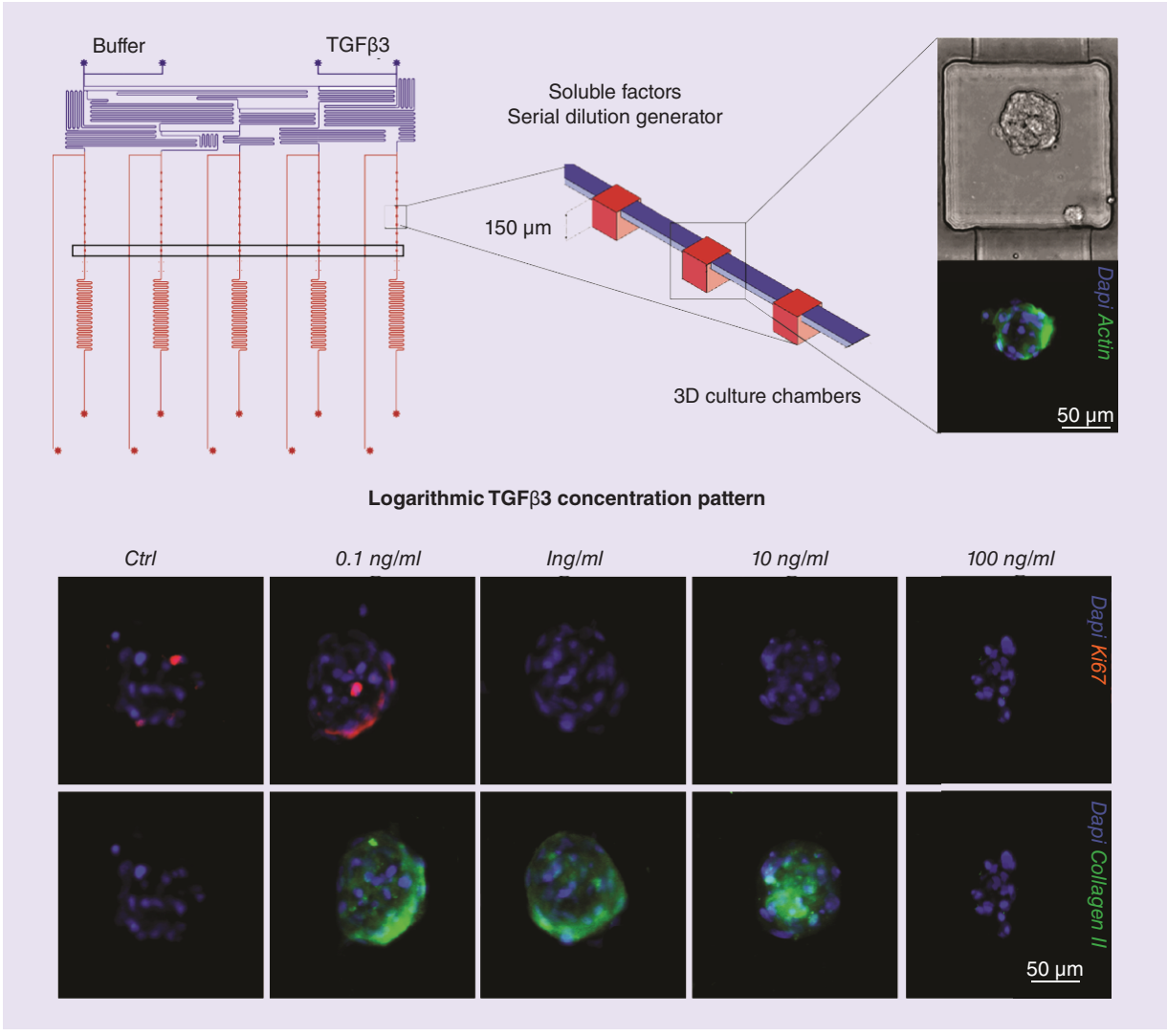


Figure 1. A microfluidic platform was introduced for 3D hMSCs micromasses generation and culturing under spatially defined patterns of soluble factors.

OP-078 Effect of mesenchymal stem cell intraperitoneal transplantation in rats after gastrotomy.

*Y. Orlova[1], Z. Abdulatipova[2], I. Trubijina[2], O. Vasnev[2], A. Luyndup[3]

[1]Science and Medical Center of Office of the President, general surgery, Moscow, Russian Federation
[2]Moscow Science and Medical Center, Moscow, Russian Federation
[3]First Moscow Medical State University, Moscow, Russian Federation

Objective

Wound healing classically comprises hemostasis, inflammation, proliferation, and remodeling. All stages are connected with each other, any disturbances on one stage holds up the beginning of the next stage. Intracellular matrix plays a coordinating role in this system, it makes a signal to anti-inflammatory cells that start to migrate to the discharging lesion. Mesenchymal multipotentional stem cells (MMSC) are one of those cells. MMSC application in regenerative field of medicine seems very perspective because of their characteristics: paracrinne effects, immunomodulation and differentiating potential. The aim of research is to establish the effectiveness and safety of MMSC application in wound healing.

Materials and methods

We used 180.0 weight white rats Wistar as the object of the study. Animals were kept in accordance with the relevant standards of GOST, at 18-20 C. We used ether anesthesia given a rate of 3-5 ml per 1 kg of body weight through a mask in a mixture with atmospheric air. Prior to gastrotomy rats were not fed for 18 hours, leaving a free access to water. Experimental animals in the amount of 40 were divided into two groups :

- 1. Test groups - 2 groups of 5 animals (10 animals).
- 2. Two series of experiments - 2 groups of 5 animals (10 goals).
- I- th series , autopsy on day 5.
- II- th series , autopsy on day 16.

A mononuclear cell fraction was aspirated from animals' tibial and femoral bone marrow. Primary cultures of predominantly mononuclear BM cells are seeded in an amount of 2,06 - 2,56cells / ml in culture dishes and cultivated till 1st and the 2nd passage. The intraperitoneal injection was administered to model rats on third postoperative day in a single dose of 2,5 106 cells. In a second series of experiment, allogeneic cells are administered to the rats twice: the first dose of 2,5 106cells on day 3 after surgery, the second injection at the same dose was carried out on day 8. There are also control series without introducing MMSC, which were injected one - or two times with physiological solution in equal volumes with the experimental series. The second control - intact animals without gastrotomy with the introduction of MMSC in the same doses written above. To verify the progress of regeneration processes and immune balance markers in the organism (cytokines), we checked concentration of TNF , IL1 , IFN , IL-4 in blood serum before surgery and during the period of wound healing.

Results

In the MMSCs transplantation group there was noticed earlier change of destructive and inflammatory phase of wound healing on proliferative phase compared to the control group. The beginning of the reparative process was indicated by more rapid sloughing. Large number of vessels and moderately severe diffuse inflammatory infiltration of segmented neutrophils were observed in the granulation tissue, and early signs of epithelialization appeared. There was an increase in the number of blood vessels, the structure of collagen fibers was more order, inflammatory infiltration was weakly expressed predominantly with lymphocytes and histiocytes.

Conclusion

1. Qualitative surgical stomach wound healing along with cytokine imbalance doesn't happen and is characterized by prolonged increase of proinflammatory (TNFα, IFNγ, IL1β) and decrease of anti-inflammatory cytokine (IL- 4).
2. Experimental evidences underscore that cytokine imbalance correction in the rats' organisms after MMSC transplantation reduces the systemic inflammatory response, inhibit immune inflammation at the local level and thereby promotes quality of the surgical wound healing.

OP-079 Surface engineered substrates for chondrogenic stem cell differentiation

*M. Öztürk[1], D. Hür[2], L. Uzun[3], B. Çelebi[3], E. Kılıç[3], D. Uçkan Çetinkaya[3], B. Garipcan[1]

[1]Boğaziçi University, Biomedical Engineering, Istanbul, Turkey
[2]Anadolu University, Eskişehir, Turkey
[3]Hacettepe University , Ankara, Turkey

Cartilage damaged by trauma has a relatively very low self-regeneration capacity due to its avascular nature. Although many repair techniques have been proposed,the future of managing cartilage defects lies in the cartilage tissue engineering. In tissue engineering, the uses of cell source, biomaterial and culture conditions have different effects on regeneration. The major drawback of cartilage tissue engineering is dedifferentiation which mainly occurs due to the used cell type and contact of cells with flat, rigid surfaces.Main objective of this study is to prepare substrates with cartilage-like chemical, mechanical and topographical surface properties to enhance a better cellular activity, to induce stem cell differentiation and to inhibit dedifferentiation of chondrocytes.

Polydimethylsiloxane (PDMS) is a biocompatible, non-toxic elastomeric polymer which is used as a cell substrate for different tissue engineering applications. PDMS has been synthesized with different prepolymer-crosslinker ratios to achieve the range of healthy human articular cartilage's stiffness (0,45-0,80 MPa). A template mimicking the collagen type II bundle alignment, in geometry and the size of healthy human cartilage tissue were prepared by photolithography. PDMS substrates with desired patterns were prepared by soft lithography. In order to mimic the chemistry of the cartilage tissue micro-environment, PDMS substrates were modified with amino acid conjugated self-assembled molecules (SAMs). Stiffness of PDMS substrates were analyzed with nanoindentation measurements and chemical modifications of substrates were confirmed by using X-ray Photoelectron Spectroscopy (XPS) and contact angle measurements.

The nanoindentation measurement confirms that the range of healthy human articular cartilage's stiffness was achieved, as the prepared substrates Young's modulus data were between 0,56±0,06 and 2,13±0,15 MPa. Surface topography of type II collagen bundles-like patterned PDMS substrates were analyzed by using optical microscopy. The results have shown that collagen type II bundle alignment was mimicked properly with PDMS. Modifications of PDMS substrates with amino acid conjugated SAMs were confirmed by using XPS analysis with sufficient surface coverage and stability. The changes in surface wettability were shown from the contact angle data after modifications of the substrates with hydrophilic/ hydrophobic amino acid conjugated SAMs. Furthermore, preliminary studies were performed using bone marrow derived mesenchymal stem cells.

According to the characterization results, prepared substrates with cartilage-like stiffness, chemistry and topography are substantial cell substrates for cartilage tissue engineering. In the ongoing studies, controlled and directed differentiation of mesenchymal stem cells on these novel substrates will be investigated in detail.

OP-080 Cationic polymers for efficient delivery of nucleic acid therapeutics for cancer therapy

*I.- K. Park[1]

[1]Chonnam National University Hospital, Department of Biomedical Sciences, Gwangju, South Korea

Objectives

The current cancer therapeutics aims for correcting the genetic anomalies responsible for vigorous cell proliferation and metastasis is by interfering with the expression of abnormal proteins. For effective gene delivery, viral vectors are unfortunately accompanied with safety issues and cytopathic effect. Therefore, synthetic non viral vectors for gene delivery have become an attractive alternative to viral vectors. Currently, we have developed and aimed to deliver a) thiolated Akt1 siRNA using disulfide crosslinked polyethylenimine (ssPEI) in CT26 colon cancer mouse model and b) miRNA 145 using polysorbitol mediated transporter (PSMT) polymer in breast cancer cells; for reducing the cell proliferation and increase apoptosis.

Material and methods

Branched PEI (bPEI) 25K and linear PEI (lPEI) 25K were pur-chased from Sigma-Aldrich. Akt1 siRNA and scrambled (scr) control siRNA were purchased from Genolution Pharmaceuticals. Propidium iodide was purchased. The CellTiter 96®AQueous Non-Radioactive Cell Prolif-eration Assay (MTS assay) was purchased from Promega and used as per the manufacturer's protocol. Synthesis of ssPEI and PSMT were performed according to the previous studies. The siRNA and miRNA are added to the polymers according to different N/P ratio. The particle size and zeta potential of the Nano complexes were measured using Zetasizer Nano Z instrument. Silencing and cell proliferation inhibition efficiency of miRNA145/PSMT is analysed in GFP expressing MCF7 cell line. CT-26 cells (5×10^5cells per mouse) subcutaneously into BALB/c mice. Tumor size was measured using digital calipers and tumor volume was calculated. 35 µg of siRNA complexed with ssPEI (SAT) at an N/P ratio of 15 was injectedon days 1 and 10 intratumorally. Akt1 knockdown in tumor tissue was confirmed by western blot analysis.

Results and conclusions

From Dynamic light scattering (DLS), Particle sizes of the Akt1 SH-siRNA/ssPEI nanoparticles (SAT) and PSMT/ pDNA were from 150 to 200 nm with a surface charge around +15 - +25 mV. Gel retardation assay showed complete DNA retention for SAT and PSMT were found at N/P 15 and 20 respectively. Both the nanoparticle have proven to be best candidate for gene delivery since they have small particle size, high postive charge and DNA complexation with less amount of polymer.

The tumor reduction study for ssPEI/SH-Akt1 siRNA (SAT) nanoparticle was performed and compared to the tumor mice treated with branched PEI (25K) complexed with SH-Akt1 siRNA (BAT). The tumor volume and size of SAT treated tumor mice is much low compared to BAT. This signifies that SAT particle efficiency transfected the tumor cells and released the siRNA for silencing Akt1 protein. Silencing of Akt1 in tumor has lead to reduction in proliferation and induced apoptosis.

The target gene c-myc protein expression was reduced as the N/P ratio of the PSMT/ miRNA 145 treatment to cells was increased compared to normal cells. With the reduction in c-myc expression, the cell proliferation of MCF7 cell lines also decreased. From our studies, we have confirmed that Akt1 siRNA and miRNA 145 delivered by ssPEI and PSMT has reduced the proliferation and induced apoptosis in cancer models both in invitro and invivo condition efficiently when compared to control carrier.

OP-081 The interaction between transplanted apical papilla stem cells and host cells in an *in vivo* pulp bioengineering model.

*C. Pelissari[1], M. Trierveiler[1], A. Mantesso[1]

[1]University of Sao Paulo Dental School, Oral Pathology, Sao Paulo, Brazil

The use of stem cells is already a reality in some areas of Medicine, but the same does not apply for Dentistry, which keeps on using artificial materials to replace lost dental tissues. Since the dental stem cells were identified, the studies in the stem cells area advanced but there is still a lack of knowledge about what these cells can do after transplantation. Thereby, this study sought to examine the co-participation of transplanted human cells and host cells in an *in vivo* model of dental pulp engineering. For this purpose a construct was built using mouse first molar empty crowns filled with PureMatrix™ associated or not to a pool population of human apical papilla stem cells (SCAPs). The constructs were transplanted using a SCID mouse kidney capsule model, in which a highly vascularized and easily accessible site with a low immunogenic response is used. First molars crowns of newborn mice were obtained and treated with EDTA 0.2% before receiving the cells and being transplanted for 21 and 28 days. Human cells were identifying by expression of lamin A protein. The morphological analysis of all transplanted crowns (including those that received PureMatrix™ only) showed formation of a loose connective tissue of variable cellularity, with the presence of well-formed vessels with erythrocytes inside. The pulp chambers of teeth that received cells usually exhibited osteodentin formation primarily associated with the dentin, organized in a concentric manner and sometimes occluding a large part of the crown space. The anti-lamin A protein reactions failed to detect positive humans cells in the newly formed tissue. In conclusion, the presence of cells is not necessary to form a connective tissue inside the pulp chamber, however they surely played some physiological function as osteodentin was only identified in the samples that received them. It is not clear if the use of cells is an advantage or disadvantage as the capacity to form osteodentin in response to injury is important but the possible obliteration of the pulp chamber is not a desirable effect.

OP-082 Prevention of GvHD by antisense mediated *ex vivo* gene therapy

*S. Przybylski[1], M. Gasch[1], M. Ebert[1], A. Ewe[2], N. Hilger[1], S. Fricke[1], A. Aigner[2], F. Emmrich[1,3], J. Burkhardt[1]

[1]Translational Centre for regenerative Medicine, University Leipzig, Leipzig, Germany
[2]Rudolph-Boehm Institute for Pharmacology and Toxicology, Clinical Pharmacology, University Leipzig, Germany
[3]Fraunhofer Institute for cell therapy and immunology, Leipzig, Germany

Introduction

Graft versus Host Disease (GvHD) is a common, severe and often fatal complication of allogenic hematopoietic stem cell transplantation (HSCT). Donor T-cells react against host antigens and initiate a massive cytokine release, which induces tissue damage in the host. By applying antisense oligonucleotides (AONs) directed against T-cell receptor genes, we aim to prevent GvHD. Transient knockdown of GvHD relevant immune cell genes allows temporary immune suppression without severe impairment of general immune functions.

In the future, this approach might also be suitable for treatment of other T-cell mediated immune diseases, such as Rheumatoid Arthritis or asthmatic disease.

Material and Methods

AONs were designed to pair mRNA encoding T-cell receptors (e.g. cd4, cd28) and synthesized as 2'O-methyl phosphorothioate RNA (2'O-Me-PTO). Transient transfection methods were established in view of effectiveness and safety with a focus on a modified polycationic polyethylenimine (PEI, AG Aigner). AONs were tested *in vitro* for effects on target gene expression and functional outcome (e.g. cytokine expression, T-cell proliferation). Most promising AONs were then applied to a murine *in vivo* GvHD animal model. Here, 10 µg AON (anti-cd4, anti-cd28, combined anti-cd4 + anti-cd28 or *nonsense* mock control) were transfected by PEI into 20x10^6 donor splenocytes (C57/Bl6) *ex vivo* and incubated for 4h prior to mixing with 20x10^6 donor bone marrow cells and injected intravenously into irradiated (0.4 Gy/g KG, max. 8 Gy) host animals (Balb/c). Host mice were then scored daily for clinical features, hemograms and blood flow cytometry (FC) were obtained bi-weekly. Upon sacrifice, tissues were preserved for RNA extraction and histological analysis.

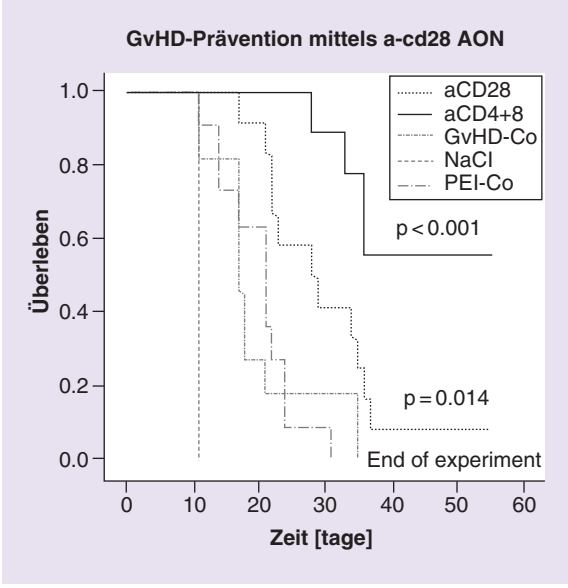


Figure 1. Survival of Balb/c recipient mice after allogeneic transplantation of 20x10⁶ bone marrow cells and 20x10⁶ splenocytes after treatment of 10 µg anti-cd4, anti-cd28, combined anti-cd4 + anti-cd28 or nonsense antisense oligonucleotides (n(anti-cd4)=30, n(anti-cd28)=7, n(anti-cd4+28)=9, n(GvHD-Co)=26 , n(PEI-Co)=26, n(NaCl)=4).

Results

In vitro transfection of AONs in murine spleen cells reduced target receptor mRNA as well as surface receptor expression significantly. *Ex vivo* application of anti-cd28 AON and a combination of anti-cd4 and anti-cd28 AON significantly increased survival ($p = 0.014$, $p < 0.001$, respectively). Engraftment of donor cells was successfully detected by FC analyses between days 5 to 12 and hemograms showed successful restoration of white blood cell count following irradiation induced decrease during the experiment. Results of real-time PCR analysis revealed reduced pro-inflammatory cytokine expression in spleen and colon of treated animals compared to controls. Histological analyses of skin and colon showed reduced GvHD-relevant inflammation in treated animals as well.

Conclusions

We conclude that *ex vivo* transfection of AONs directed against T-cell receptors improved tolerance of regenerative HSCT and might enhance the range of this therapeutic option. In the future, AONs might also be applied to treat GvHD by direct *in vivo* application and this immune

OP-082

suppressive technique might even be transferred to other immune diseases with similar disease pathology (e.g. RA, MS, asthma).

Thus, we propose a new class of immunosuppressive drugs based on AONs directed against T cell receptor genes.

Disclosure

This work was supported by funding from the German Federal Ministry of Education and Research (BMBF 1315883), by the Sächsisches Staatsministerium für Wissenschaft und Forschung (SMWK; Project "Verfahren zur effizienten, Nanopartikel-vermittelten Einschleusung therapeutischer Nukleinsäuren in Zellen des Immunsystems zur Gentherapie immunologischer Erkrankungen") and by the Translational Centre of regenerative Medicine (TRM; Project R4029).

OP-083 Functional measurements of electrolyte transport involved in pH regulation and bicarbonate secretion of HAT-7 ameloblast cells

*R. Rácz[1], E. Bori[1], P. DenBesten[2], A. L. Bronckers[3], M. C. Steward[4], G. Varga[1]

[1]Semmelweis University, Department of Oral Biology, Budapest, Hungary
[2]University of California, San Francisco, Department of Orofacial Sciences, San Francisco, United States
[3]ACTA, Department of Oral Cell Biology, Amsterdam, Netherlands
[4]The University of Manchester, Faculty of Life Sciences, Manchester, Great Britain

Objectives

The hardest mammalian tissue, dental enamel is produced by ameloblasts, which are electrolyte-transporting epithelial cells. During amelogenesis the formation and growth of hydroxyapatite crystals generates a large number of protons that must be neutralized, presumably by bicarbonate ions transported from ameloblasts into the developing enamel matrix. Buffering requires a tight pH regulation and secretion of bicarbonate. The whole process has been the focus of many immunohistochemical and gene knock-out studies, but no functional data exist for mineral ion transport by ameloblasts. Therefore, we developed a novel cellular ameloblast model to investigate these mechanisms using the polarized HAT-7 rat ameloblast cells grown on Transwell permeable support. In the present work we aimed to provide functional evidence for the role of several key electrolyte transporters in our model. Additionally we tested whether HAT-7 cells achieve transepithelial bicarbonate transport under our experimental conditions.

Materials and methods

To obtain monolayers, HAT-7 cells were seeded on Transwell membranes and cultured in differentiation medium for 4 days. We monitored transepithelial electrical resistance (TER) as an indicator of tight junction formation and polarization. We evaluated intracellular pH changes by microfluorometry using BCECF fluorochrome. The activity of anion exchangers (AE) were tested by withdrawal of chloride ions and using anion exchange inhibitor DIDS. With ammonium-pulse technique, we inspected the compensation after a rapid intracellular alkalization/acidification by withdrawal and subsequent restoration of sodium ions. The activity of sodium/proton exchanger (Nhe) was investigated by its inhibitor amiloride in a bicarbonate/CO_2-free solution. Sodium/potassium/chloride cotransporter (Nkcc) activity was also tested in this setting by its inhibitor bumetanide.

Results

HAT-7 cells formed epithelial layers with measureable TER on Transwell permeable supports. We detected Nhe activity, a sodium-dependent amiloride-sensitive compensation after acidosis, at the basolateral side of HAT-7 cells (0.326±0.026 vs 0.047±0.005 ΔpH/min, $p<0.001$, control vs inhibited). We found basolateral, DIDS-sensitive anion exchanger activity, most probably Ae2 (0.141±0.024 vs 0.051±0.012 ΔpH/min, $p<0.005$), and basolateral Nkcc activity, very likely due to Nkcc1 (-0.084±0.004 vs -0.057±0.003 ΔpH/min, $p<0.001$). A detectable vectorial (basolateral to apical) bicarbonate transport was observed by simultaneously inhibiting basolateral NHE activity with amiloride, and sodium-bicarbonate cotransport activity with H_2DIDS. Measurements of transepithelial HCO_3^- transport showed a marked increase in response to Ca^{2+}- and cAMP-mobilizing stimuli (ATP and forskolin, respectively).

Conclusions

We could verify the activity of several key transporters affecting the pH regulation of HAT-7 ameloblast originated cells. HAT-7 cells are functionally polarized and can accumulate HCO_3^- ions from the basolateral side and secrete them at the apical membrane. Our HAT-7 model provides a useful tool for better understanding of amelogenesis and may serve in the future for tissue engineering efforts to rebuild lost or damaged dental enamel.

Disclosure

Supported by NIH NIDCR 5R01DE013508 subaward: 7743sc, by the Hungarian National Research Development and Innovation Office (TÁMOP-4.2.1/B-09/1/KMR-2010-0001 and OTKA-NKTH CK-80928).

OP-085 Activation of the aryl hydrocarbon receptor modulates *ex vivo* murine myelopoiesis

*S. Riemschneider[1], J. Kohlschmidt[1], J. Lehmann[1]

[1]Fraunhofer Institute for cell therapy and immunology, GLP cell engineering, Leipzig, Germany

Objectives

The aryl hydrocarbon receptor (AhR) is involved in a variety of cellular processes including detoxification, liver homeostasis and circadian rhythm. Furthermore, the role of AhR in the immune response as well as its influence on T cell development was highlighted in numerous studies during the last decade. In contrast, the involvement of AhR in hematopoiesis of myeloid cells is still poorly understood. Therefore our study focused on the influence of AhR activation on the proliferation and differentiation of bone marrow-derived myeloid cells.

Materials & methods

Cells were isolated from bone marrow of *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ C57BL/6 mice and cultured in medium with M-CSF-enriched supernatant from L929 cells to induce the differentiation into macrophages. Cells were then exposed to a subtoxic dose of the AhR ligand benzo[a]pyrene (BaP) or to the natural ligand indole-3-carbinol (I3C) found in cruciferous vegetables. After 6 days of incubation, expression of myeloid cell surface markers was analysed by flow cytometry to characterise the subpopulations of differentiated myeloid cells.

Results & conclusion

Both AhR ligands were able to inhibit cell proliferation of bone marrow cells, an effect that was absent in cells derived from *Ahr*$^{-/-}$ mice. With respect to different myeloid subpopulations, we observed that the differentiation of specific cell populations was influenced by AhR activation. Immature myeloid cells characterised by the presence of CD11b^{+}/Gr-1^{+} and the absence of markers for mature myeloid cells (i.e. F4/80, CD11c, CD64, CD14, MHC-II) were arrested in their premature state by AhR activation. Therefore, the number of differentiated macrophages characterised by the surface markers CD11b and F4/80 was reduced. In contrast, the expression of both F4/80 and Gr-1 was increased on these adherent macrophages. Additionally, a small macrophage-like population with a higher expression of CD11b and Gr-1, characteristic for monocytic myeloid-derived suppressor cells, could be detected. In contrast to the blocked differentiation of macrophages, the development of this macrophage-like cell population was enhanced by AhR activation. Furthermore, we observed a small population of dendritic cells, characterised by the expression of CD11c^{+}/MHC-II^{+}/CD80^{+} that was also induced by AhR activation. The modulation of myelopoiesis by BaP and I3C was absent in *Ahr*$^{-/-}$ cells indicating that the effects were dependent on AhR activation. Our results imply that AhR is involved in proliferation of hematopoietic stem cells and particularly in the differentiation of myeloid cells.

OP-087 Novel bioreactor system for culture of tissue engineered heart valves

*J. Saam[1], H. Erfurth[1], C. König[2], A. Schill[2], C. Lüders[3]

[1]OSPIN GmbH, Berlin, Germany
[2]Charite - Universitätsmedizin Berlin, Centrum Wissenschaftliche Werkstätten (CWW), Berlin, Germany
[3]Deutsches Herzzentrum Berlin, Labor für Tissue Engineering, Berlin, Germany

Approximately 275,000 patients receive a heart valve replacement annually which are limited to either a metal valve replacement or a preserved (typically allogeneic or xenogeneic) tissue valve replacement. Although the function of these conventional replacements is satisfying, patients are subject to the morbidity associated with anticoagulation when mechanical valves are used or the limited durability of a biological prosthesis with the prospect of replacement surgery. To overcome the limitations tissue engineered heart valves (TEHVs) could be an attractive alternative. This study is focused on the conceptual design and the establishment of a specific bioreactor system for the automated generation of TEHVs mimicking the healthy native original.

A system for heart valve tissue engineering should facilitate cell seeding, cultivation and functional testing. Since many patients in need of heart valve replacement also suffer from hypertension the bioreactor should be able to train and test the TEHV under physiological as well as pathological conditions. Many existing heart valve bioreactors are not capable of tightly controlling the pressure waveform and setting certain desired conditions involves tedious manual adjustment. To address this problem we developed a computer controlled bioreactor that can produce freely programmable pressure wave forms while real time electronic control eliminates the need for large compliance chambers. The user friendly interface allows the definition of parameters in medical terms such as diastole, systole, heart rate for each process phase and supports smooth ramping between different set values. Dissolved oxygen, glucose and pH are constantly monitored. Automatic media exchange and glucose feeding is available. Homogenous seeding is achieved by slowly rotating the heart valve. Inner and outer surface of the TEHV can be seeded separately without mixing the cells. The bioreactor design eliminates the need for assembling and mounting the bioreactor under a sterile bench. Instead the entire fluid path can be sterilized with mounted heart valve scaffold and then used a closed sterile system.

To demonstrate the bioreactor system it was used to produce a tissue engineered heart valve. To this end a decellularized porcine aortic heart valve was placed in the rotatable heart valve holder and mounted in the tissue chamber. The fluid path assembly with tissue chamber and scaffold was plasma-sterilized and all openings closed while still in the sterile packing. This closed system could then be installed on its control unit and placed inside an incubator. After automatic filling with cell culture medium the valve was seeded with a predefined amount of human myofibroblasts and subsequently with endothelial cells while slow rotation of the valve ensured homogenous cell distribution. In the following phase pulsatile pressure- and flow conditions were slowly ramped up until physiological conditions were reached. In the last step the tissue construct was successfully cultured for 14 days under physiological perfusion.

Disclosure

Authors Jan Saam and Hendrik Erfurth have the following financial involement: Both are shareholders of OSPIN GmbH which performed parts of the research presented in the abstracts.

This project on which this report is based was funded by the German Federal Ministry of Education and Research, funding code 13GW0067A.

OP-092 Raman spectroscopy: a novel tool for label-free and non-invasive cell analysis and quality assurance in 3D-skin grafts

H. Kremling[1], *K. Schütze[1], D. Marino[2], S. Meyer[2]

[1]CellTool GmbH, Bernried, Germany
[2]University Children`s Hospital, Tissue Biology Research Unit, Department of Surgery, Zürich, Switzerland

Objectives

Today, regenerative medicine allows the formation of highly complex autologous skin grafts for the treatment of patients with conditions like heavy burns, giant nevus or debilitating scars. Current in-process GMP quality controls are, however, suboptimal. In fact they are time consuming, expensive, and demanding for high amounts of human specimens. Furthermore, Most of these controls cannot be technically performed on the final product.

Raman spectroscopy (RS) is a highly sensitive analytical method for marker-free and non-invasive identification and characterization of cells. Here, we wanted to demonstrate the feasibility of RS in 3-dimensional set-ups such as differentiated human gingival fibroblasts cultured within collagen matrices (mucoderm®) and autologous dermo-epidermal skin grafts derived from human skin. We could provide evidence that RS can discriminate matrix-cultured normal versus differentiated fibroblasts and skin-graft cultured fibroblasts, melanocytes and keratinocytes, even in a depth of 200µm and more.

Material & Methods

Human gingival fibroblasts (ProVitro) were cultured for 6 weeks on a 1-2 mm thick collagen matrix (mucoderm®). For activation, cells were incubated with differentiation medium (ProVitro) for 7 days. Afterwards, samples were fixed with 4% paraformaldehyde for analysis with Raman spectroscopy.

The skin grafts were cultivated with human fibroblasts, keratinocytes and melanocytes for 10 days. Subsequently, grafts were fixed with 4% paraformaldehyde for analysis with Raman spectroscopy.

Raman measurements were carried out with the BioRamÒ system (CellTool GmbH, Bernried). At least 60 cells of each group were measured and compared. Data processing was performed with customized BioRamÒ-software followed by statistical Principal Component Analysis (PCA) to find the major differences between differentiated and undifferentiated fibroblasts.

Results and Conclusion

RS could clearly distinguish differentiated from undifferentiated fibroblasts, where PCA analysis identified collagen type I, proteins and lipids as major key molecules for cell discrimination.

Also in the skin-graft setup, RS was suitable to differentiate between different cell types. The penetration depth in both setups was up to 200µm, still resulting in meaningful spectra where microscopic observation was no longer possible.

These two examples show Raman spectroscopy as suitable gentle yet highly specific detection method for cells in engineered tissue. As label-free and non-invasive method, it provides highly specific molecular information about the entire metabolome of a single cell even in a matrix setup with a depth of 200µm that is as characteristic as a “fingerprint”.

Most importantly, RS can be used for quality assessment of cell cultures or engineered tissue without impairing cell viability.

Disclosure

This project has received funding from the European Union’s Seventh Program for research, technological development and demonstration under grant agreement No 279288.
Karin Schütze und Heidi Kremling are employees at CellTool GmbH, Bernried.

OP-095 Enhanced donor and recipient vessel formation through co-transplantation of placental derived endothelial colony forming cells with various mesenchymal stem cells in the wild type and immunodeficient mice

*A. Shafiee[1], J. Patel[1], N. Fisk[1], K. Khosrotehrani[1]

[1]Institute of Health and Biomedical innovation, Queensland University of Technology, Brisbane, Australia

Objective

To assess whether co-transplantation of ECFC with mesenchymal stem cells (MSC) could improve their viability and function, cells were implanted into the wild type (WT) (C57BL/6) and immunodeficient (Rag1(-/-)C57BL/6) mice.

Methods and Results

GFP-tagged ECFC implantation in matrigel plugs showed most cells undergoing death 1 week after implantation. Although implantation in WT mice resulted in 5 fold less cell engraftment compared to implantation in RAG1(-/-) mice ($P < 0.01$). In RAG mice, co-implantation with adult bone marrow (AdBM-MSC), fetal bone marrow (fBM-MSC), fetal placental (fPL-MSC), or adult placental (AdPL-MSC) resulted in a significant increase in cell engraftment reaching from 96-205 fold. fPL-MSCs had a slightly higher potential to promote ECFC engraftment. In WT mice, MSC co-implantation also had a significant but smaller effect on ECFC engraftment ranging from 0.2-11 fold. AdBM-MSC co-transplantation had the best potential to increase human vessel density in implantation area for 21 ($P < 0.001$) and 3.5 ($P < 0.05$) fold in RAG1(-/-) and WT mice respectively. Additionally, AdBM-MSC enhanced the mouse vascular area 3 and 4.3 fold in RAG1(-/-) and WT mice respectively ($P < 0.05$). Moreover, co-transplantation improved murine vascular formation when injected into excisional skin wounds after 7 days in both strains. Culture experiments recapitulated *in vivo* findings.

Conclusion

Co-implantation of MSCs with PL-ECFCs resulted in improved survival and function in both hosts which depends on the MSC source and host immune system. Together, MSC and ECFC co-implantation approach provides an essential step towards clinical application of ECFCs in allogeneic recipients.

OP-096 New approaches to increase functional activity of endogenous stem cells for regenerative medicine

*E. Skurikhin[1], O. Pershina[1], N. Ermakova[1], V. Krupin[1], A. Pakhomova[1], A. Dygai[1]

[1]Research Institute of Pharmacology and Regenerative Medicine name after ED Goldberg, Tomsk,, Russian Federation

Objective

Last years, the use of stem cells has given rise to many hopes in regenerative medicine, especially in diseases without efficient therapies. Clinical trials on the use of stem cells are underway for a wide variety of conditions and there is an emphasis on the use of bone marrow, hematopoietic (mobilized and recovered in blood and umbilical cord blood) and mesenchymal stem cells. The attention is focused on using of stem cells for tissue regeneration. According to modern concepts stem and progenitor cells are localized in many organs and tissues (bone marrow, pancreas, lungs, etc.) of an adult organism, and have a high potential to self-renew, depending on the localization they differentiate into cells of hematopoietic, mesenchymal lines or other specialized cells. Many researchers suggest to use this suspected internal reserve in the treatment of serious chronic degenerative diseases. Currently there is no understanding the behavior separate subpopulations of regional adult stem cells *in vivo* and disease. This hampers the development of pharmacological modulation of adult stem cells. From our point of view, a safe treatment for many chronic diseases compared with transplantation of Stem cells may be the use of endogenous adult stem cells. In our opinion, pharmacological of modulation adult stem cells may be effective. A search of compounds that modify the function of endogenous stem and progenitor cells is very important for regenerative medicine.

Methods

Experiments were performed on C57BL/6, BALB/C and CBA mice. All our experimental procedures with animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. We investigated the effect of neurotropic drugs, enzymes altering the extracellular matrix, plant product and pharmacologically active molecules on carriers on stem and progenitor cells derived from bone marrow, lung, pancreas and mammary gland at the experimental models of leukopenia, diabetes mellitus, fibrosis and breast cancer. Our investigations were performed with a strict methodology and compliance with world standards. In our research we used the histological methods for investigation of tissues. We evaluated by flow cytometry content of HSCs, pan-hematopoietic cells, hematopoietic progenitor cells and MSCs derived from different tissues. We evaluated the mobilization, migration, engraftment after transplantation, proliferation, differentiation and regenerative potential of endogenous stem and progenitor cells.

Results and conclusions

Each disease has a specific activity of endogenous stem cells. From our point of view, influencing stem cells and progenitor cells chronic diseases can be treated.

We would like to demonstrate dates by our own studies of compounds on models of the pneumofibrosis, diabetes, myelosuppression and breast cancer.

As result of our investigation we identified target-cells for each group of pharmacologically active molecules. We proposed new approaches for inhibition of inflammation and fibrosis and stimulation of tissue regeneration. We made overall conclusion these approaches can be proposed for implication in regenerative medicine.

OP-097 The influence of activated autologous platelet-rich plasma (PRP) and hydroxyapatite tricalcium phosphate on bone defects healing in experiments carried out on animal models

*S. Skwarcz[1], A. Gregosiewicz[2], I. Bryzek[3], J. Skwarcz[4], R. Nadulski[5], J. Sanoford[6]

[1]Medical University of Lublin, Department of Orthopaedics and Traumatology, Lublin, Poland
[2]Medical University of Lublin, Department of Paediatric Orthopaedics, Lublin, Poland
[3]Veterinary Clinic – Bryzek and Partners, Lublin, Poland
[4]University of Life Sciences, Department of Technology Fundamentals, Lublin, Poland
[5]University of Life Sciences, Department of Food Engineering and Machinery, Lublin, Poland
[6]Vetregen, Warsaw, Poland

Objectives

The purpose of this study is to evaluate the effect of autologous platelet-rich plasma (PRP) and tricalcium phosphate (80%) hydroxyapatite (20%) (BoneSave - Stryker) on supporting the healing of unicortical experimental bone defects in rabbit femoral bone and to assess the suitability of research methods: classical radiology, densitometry, micro-CT, biomechanical tests and histological evaluation.

Material and methods

36 Termond White Rabbits used in the paper were divided into 3 groups (A, B, C) of 12 animals each. In all groups (A, B, C) tested substances were placed in the experimental defects in right femoral shafts (marked ”P” in the paper), while the left femur (marked “L” in the paper) constituted a control group, in which produced defects were left unfilled. In group A, tricalcium phosphate (80%) hydroxyapatite (20%) (BoneSave - Stryker) at 100 mg was given to the right femoral marked “P” in place of the produced defect. In Group B, activated autologous platelet rich plasma (PRP) in an amount of 0.7 ml was given to the right femoral marked “P” in place of the produced defect. In group C tricalcium phosphate (80%) hydroxyapatite (20%) (BoneSave - Stryker) in an amount of 100 mg soaked in 0.7 ml of the activated autologous platelet rich plasma (PRP) was given to the right femoral marked “P” in place of the defect. At the beginning and the end of the experiment, blood was drawn for laboratory tests and the level of platelets in peripheral blood and in the preparation of the PRP, the level of osteocalcin and bone isoenzyme of alkaline phosphatase were determined. In all groups the experiment finished after 8 weeks after the surgery. After killing the animals femoral bones were collected and evaluated: radiologically (X-ray performed every 7 days), densitometrically, morphologically, micro-CT, biomechanically (three-point test) and histologically.

Results and conclusions

1. Hydroxyapatite tricalcium phosphate stimulates the bone formation in the bone defects area in experimental animals in radiographic evaluation, macroscopic measurements, micro-CT and histopathological evaluation. **2.** Autologous activated platelet rich plasma used as the only filling of experimental bone defects: **a.** stimulates new bone formation in the area of application in radiographic evaluation, macroscopic measurements, micro-CT and histological evaluation; **b.** weakens bone biomechanical properties in the tested phase of healing. **3.** Autologous activated platelet rich plasma combined with hydroxyapatite tricalcium phosphate: **a.** has a favourable effect on the mineralization of the newly created bone tissue increasing the amount of the fraction of higher density in a total volume of bone; **b.** does not improve the densitometic parameters, biomechanical properties, and the total volume of bone tissue in the defect area in comparison with the isolated treatment with bone substitute. **4.** Differences in concentrations in bone isoenzyme of alkaline phosphatase and osteocalcin in the blood serum of experimental animals cannot be treated as laboratory indices of dynamic of healing of experimental bone defects. **5.** The use of micro-CT studies in the analysis of bone defect healing is a useful tool in the qualitative and quantitative assessment of bone volume changes. **6.** The research on the effects of PRP on bone tissue healing requires standardization of methods of preparation and administration of PRP and the development of research models to enable repeatable assessment of their effectiveness.

OP-099 Functional dissection of genes employing esiRNAs and esiCRISPR in mouse embryonic stem cells

*I. Steinebrunner[1], M. Theis[1,2], L. Ding[2], M. Schneider[2], F. Buchholz[2]

[1]Eupheria Biotech GmbH, Dresden, Germany
[2]Technische Universität Dresden, Medical Systems Biology, Dresden, Germany

RNA interference (RNAi) through short interfering (si)RNAs is an effective method to unravel gene function in a wide variety of model systems. However, the use of single siRNAs is frequently accompanied by off-target effects.

In order to tackle this problem, we have developed a different RNAi technology. Instead of using single siRNAs to silence a target, a long double-stranded (ds) RNA matching about 500 bp of the target sequence is digested by endoribonuclease into a pool of highly diverse siRNAs termed esiRNAs. Due to the sequence diversity of the esiRNAs off-target effects are avoided, while the pool of siRNAs synergistically contributes to efficient silencing of the intended transcript [1]. Hence, esiRNAs offer efficient knockdown at superior specificity.

esiRNAs have demonstrated their utility in numerous genome-wide screens and are now used by many researchers around the world to investigate gene function of protein-coding and non-coding transcripts [2–4]. Here, we present esiRNA-based genome-wide screens comparing mouse embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs). Both cell types are pluripotent, but ES cells are derived prior to the implantation stage, while EpiSCs thereafter. Oct4 is a key regulator of pluripotency in both cell types, and a GFP reporter line was used for each stem cell type to uncover genes required to maintain cell identity. The screen delivered numerous genes that are required for sustained Oct4 expression in both ESCs and EpiSCs [5]. However, the screen also unmasked fundamental differences of Oct4 regulation in ESCs and EpiSCs, which will be presented during the talk. We will also present our latest developments to utilize the CRISPR/Cas9 technology for gene function analysis and validation of RNAi phenotypes.

Conclusions

esiRNAs are efficient and specific mediators of RNAi and reduce false positives in genome-scale screens due to their high specificity. esiRNA-based screens comparing ES cells and EpiSCs confirmed the reliability of the technology and unearthed a fundamental difference in the regulation of Oct4 between the two pluripotent stem cell types. We further show that gRNA/Cas9 ribonucleoprotein complexes can be directly delivered into cells to probe gene function and to perform validation experiments.

Disclosure

Financial support was received from EU FP7 grant SyBoSS (242129) awarded to FB, DFG grants BU 1400/3-1 and BU 1400/5-1 awarded to FB and LD, and DFG grant SFB655 (B5) awarded to FB. IS, MT, FB are affiliated with Eupheria Biotech.

References

1 Kittler R1, Surendranath V, Heninger AK *et al.* Genome-wide resources of endoribonuclease-prepared short interfering RNAs for specific loss-of-function studies. *Nat. Methods*, 4, 337–344 (2007)

2 Raychaudhuri S, Loew C, Körner R *et al.* Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. *Cell* 156. 975–985 (2014).

3 Chakraborty D1, Kappei D, Theis M *et al*. Combined RNAi and localization for functionally dissecting long noncoding RNAs. *Nat Methods* 12, 360–362 (2012)

4 Theis *et al. J. Biomol. Screen.* (2015) (In Press).

5 Ding *et al. Cell Systems* (2015) (In Press).

OP-101 *In vitro* study of human osteoblast-like MG-63 cell line on the bio-ceramic nano-powders

M. Markova[1], *T. Suchy[2,3], E. Filova[1], J. N. L. der Kinderen[4], Z. Sucharda[2], M. Supova[2], K. Balik[2], L. Bacakova[1]

[1]Czech Academy of Science, Institute of Physiology, Prague, Czech Republic
[2]Czech Academy of Sciences, Institute of Rock Structure and Mechanics, Prague, Czech Republic
[3]Czech Technical University in Prague, Faculty of Mechanical Engineering, Prague, Czech Republic
[4]Rijksuniversiteit Groningen, Groningen, Netherlands

Question

Hydroxyapatite is an anorganic part of bone matrix, which has a biomimetic effect on cells. Physical and chemical properties of hydroxyapatite, such as solubility, size, and shape depend on the method of preparation. In this study nano-hydroxyapatite of both biological and synthetic origin were evaluated *in vitro* with osteoblast-like MG-63 cells as novel biocompatible and bioactive materials for regenerativemedicine.

Methods

MG-63 cells were seeded into cell culture dishes with the following nano-powders: bovine bone bio-apatite (B-Cap), prepared by calcination at 500°C, 600°C or 700°C, fluorinated calcium phosphate (F-Cap), magnesium-doped brushite (Mg-Cap), calcium-deficient hydroxyapatite (CDHA), and commercially available tricalcium phosphate (TCP) and hydroxyapatite (HA), and cultured for 7 days. The cell viability and cell numbers were assessed by XTT assay, by a LIVE/DEAD® Viability/Cytotoxicity Kit, by Hoechst & Texas red staining, and by an xCelligence assay. Immuno-fluorescence staining of β-actin, vinculin, osteocalcin, and osteopontin was performed as well.

Results

We observed that both bio-apatite and commercially available synthetic ceramic powders supported the adhesion and growth of MG-63 cells. The B-Cap 700°C and F-Cap showed higher colonisation with MG-63 cells than synthetic HA and TCP. We also found out that in medium with B-Cap 600°C and HA the concentration of Ca2+ was decreased, which could impair cell growth.

Conclusion

Both B-Cap 700 °C and F-Cap seem to be most appropriate materials for cell growth. Generally, the bio-ceramics has a potential importance for bone tissue engineering.

OP-102 Strategies for automated ATMP production

*A. Traube[1], J. Kopf[1], A. Börner[1], A. Traube[1]

[1]Fraunhofer IPA, Laboratory Automation and Biomanufacturing Engineering, Stuttgart, Germany

Objective

Novel cell-based therapies have the potential to revolutionize treatment for various medical disorders. Worldwide, numerous clinical trials are ongoing; still only 13 cell-based therapies have been approved for patient treatment to date [1,2]. The manual production of cell-based therapies, resulting in immense costs, not only hampers product development but also limits accessibility to only a small group of patients. Therefore our objective was to elucidate how high numbers of individualized therapies can be produced in an economical way.

Material and Methods

A conceptual study on the technology transfer of ATMP production processes to a new production concept, including next generation automation solutions, was performed. Pivotal requirements that enable widespread commercialization and manufacturing have been analyzed and prioritized.

Based on an immune cell product, all manufacturing steps were analyzed and performance specifications, such as the in- and output format, specifications for hardware, software, process control and QC criteria were defined.

Upon definition of individual process steps and the evaluation of their automation potential conceptual solutions for hardware equipment were drafted. Widespread use automated platforms require a flexible, modular setup with standardized communication interfaces. To enable module connection a prototype for an encapsulated interface, which meets the closed-system-principle was designed. Additionally, data management and transfer as well as concepts for overall control and process scaling were implemented.

Results

Analysis of performance specification resulted in a detailed process flow chart with pivotal information concerning process steps and parameters, process flexibility, effects on product quality, and regulatory aspects. The combined analysis of all process specifications and requirements for commercialization resulted in two different manufacturing concepts.

Concept 1 is based on a closed disposable system, integrating all main functionalities such as liquid handling and cell incubation within the disposable. The disposable consists of different product containers that can be individually and serially combined into process modules. Due to its standardized design, handling can be performed with industrially established handling systems.

Concept 2 illustrates a modular production platform, consisting of different closed and independent modules for each process step, and additionally a transfer, storage and incubation system. GMP compliant clean room environments are within one process module. This similarly allows processing of several patient products on the same platform while being open for the integration of different technologies that are already commercially used for ATMP production.

In both concepts novel standardized interfaces play crucial roles to enable product transfer and stepwise automation and production in semi- or fully automated closed systems. Further technical and organizational measures have been taken to ensure strict product batch separation during scale-out. Innovative in process controls are further part of all production processes.

Conclusions

Similar to the automated production that once revolutionized the automobile industry through mass production, the use of automation solutions in the production of cell-based therapies has the potential to foster therapy development and promote availability to a wider public.

References

1 www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/default.htm

2 www.pei.de/DE/arzneimittel/atmp-arzneimittel-fuer-neuartige-therapien/atmp-arzneimittel-fuer-neuartige-therapien-inhalt.html

OP-103 Stages of ageing skin regeneration by means of autologous fibroblasts application

*V. Tsepkolenko[1,2], A. Tsepkolenko [3]

[1]Virtus, Dermatology, Odessa, Ukraine
[2]Virtus , Odessa, Ukraine
[3]Institute of Plastic Surgery Virtus, Dermatology, Odessa, Ukraine

One of the priorities of modern Aesthetic Medicine (AM) development is finding and starting a practical application of new methods and techniques to intensify skin regenerative processes.

Development of an application algorithm of using cutting edge techniques for intensive skin regeneration is considered to be an important landmark in the course of AM progress. The scheme is designed to increase moisture content in dermal ground substance, adequate skin stimulation based on its physiological capabilities, aiming to reconstruct the skin's 3-dimensional organization and qualitative and quantitative fibrous structure parameters, as well as to increase proliferating activity in fibroblasts. The scheme includes four important components (each of which has its own indications): revitalization (use of combinatorial medication containing hyaluronic acid and sodium salt of succinic acid -" Hyalual" ™); application of autologous plasma, enriched by growths factors; application of human placenta products; and fractional photothermolysis. In case on insufficient results patients were treated with autologous fibroblasts.

We have studied the effectiveness of the combinatorial approach that consists of the above mentioned components, from the perspective of evidentiary medicine. The study group included 108 patients between 41 and 71 y.o. with involutional-dystrophic skin changes. Ultrasound examination of all the patients demonstrated improvement of such objective indications as greasiness, hydration, electro-cunductivity and echodensity.

The combinatorial approach is highly effective and available to most AM doctors as a convenient technique for intensive skin regeneration without causing any side effects when properly executed.

OP-104 Bioorthogonal approaches in the development of soft materials for tissue and cell culture applications in regenerative therapies

*M. Tsurkan[1,2], J. Teichmann [1,3], R. Wetzel [4], K. Chwalek [5], A. Kozlova [4,6], R. Selzer [1], M. Binner [1], H. Pérez-Hernández [7], U. Freudenberg [1,7], C. Werner [1,2]

[1]Leibniz Institute of Polymer Research IPF, Max Bergmann Center of Biomaterials MBC, Dresden, Germany
[2]Dresden University of Technology, Dresden, Germany
[3]Dresden University of Technology , Institute of Anatomy, Medical Faculty Carl Gustav Carus, Dresden, Germany
[4]German Center for Neurodegenerative Diseases (DZNE), Dresden, Russian Federation
[5]Tufts University, Medford, United States
[6]St. Petersburg State University, Department of Chemistry, Sankt-Petersburg, Russian Federation
[7]Fraunhofer Institute for Material and Beam Technology IWS , Institute of Manufacturing Technology, Dresden, Germany

Background and Objective

Multi-parameter matrices allowing for the precise control of biochemical properties independently of mechanical network parameters are crucial in the advent of successful engineering of artificial biological systems [1]. Among them, glycosaminoglycan (GAG)-based hydrogels has attracted increasing interest for development of engineered tissues due to their unique capability of resembling the physicochemical properties of natural tissues. Bioactive molecules such as adhesive peptides, growth factors or fluorescent labels can be conjugated to GAGs implementing new properties beyond their natural functions. Introducing orthogonal strategies, in which formation and functionalization of materials can be performed independently of each other, could provide a far-reaching control over the decoupling implemented bioactivities while keeping the physicochemical properties of the material unaffected. In this work we aim to overview our recently developed multi-component hydrogel material platform which as a tool can be customized to support specific applications in both biological and medicine applications.

Material and Methods

Carbodiimide chemistry was utilized to functionalized the hydrogels with fluorescent labels (Alexa 488, Atto 532) [2] and Michael addition was utilized for hydrogel functionalization with MMP biodegradable or cell adhesive (RGD, IKVAV, GLOGER) peptides [3]. The Michael addition and carbodiimide chemistry were used orthogonally and the purities of the products were shown by chromatography. Growth factors were attached to hydrogel through physisorption. Nd:YAG laser equipped with micro lens array was utilized for the hydrogel micro structuring [4]. Sheered stress was utilized for the creation hydrogel micro-particles aggregates with defined injectable properties for *in vivo* applications [5]. *In vitro* biological response was shown on primary human umbilical vein endothelial cells (HUVEC) and isolated from hippocampus primary mice neural precursor cells (NPC) which were analysed by immunocytochemistry.

Results and Conclusion

The orthogonal approach in "initial modification" where the material precursors is functionalized prior the hydrogel formation and "post modification" where the hydrogel is formed and, afterword, is functionalized were successfully shown on the examples of four-arm polyethylene glycol with Heparin, Chondroitin and Hyaluronic acid which represent the majority GAGs of mammalian tissues. The proposed technique allows for precise control over the concentration of the integrated bioactive peptides as well as fluorescent labels which were utilized to show how the quality and quantity of adhesive ligand modulate HUVEC morphogenesis and growth rate. The proposed material strategy was also used to study the impact of geometrical confinement and biophysical properties of the cellular microenvironment on the response of NPC. In this study, precisely adjusted topography of the utilized hydrogel materials was achieved by specific light degradation which was orthogonally implemented to the cell induced degradation properties.

The concept reported here provides an innovative orthogonal methodology for customization of biohybrid hydrogel materials and allows quantifying the influence of various exogenous signals independently. As we show, this technique could be similarly applied *in vitro* and *in vivo* studies by offering a versatile tool for cell and tissue manipulations.

OP-104

References

1 Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering.. *Nat. Biotechnol*. 23, 47–55 (2005).

2 Tsurkan MV, Chwalek K, Prokoph S *et al.* Defined polymer-peptide conjugates to form cell-instructive starPEG-heparin matrices in situ. *Adv. Mater.* 25, 2606-2610 (2013).

3 Tsurkan MV, Chwalek K, Schoder M, Freudenberg U, Werner C. Chemoselective peptide functionalization of starPEG-GAG hydrogels. *Bioconjugate Chem*. 25, 1942-1950 (2014).

4 Tsurkan MV, Hauser PV, Zieris A *et al.* Growth factor delivery from hydrogel particle aggregates to promote tubular regeneration after acute kidney injury. *J. Control. Release* 167, 248-255 (2013).

5 Tsurkan MV, Wetzel R, Pérez-Hernández HR *et al.* Photopatterning of multifunctional hydrogels to direct adult neural precursor cells. *Adv. Healthc. Mater*. 4, 516-521 (2015).

OP-105 Monitoring regeneration of the axolotl nervous system using label-free multiphoton imaging

*O. Uckermann[1], J. Wohlers[1], R. Later[1], R. Galli[2], E. Koch[2,3], G. Schackert[1], E. Tanaka[3], G. Steiner[2], M. Kirsch[1,3]

[1]University Hospital Dresden, Neurosurgery, Dresden, Germany
[2]University Hospital Dresden, Clinical Sensoring and Monitoring, Department of Anesthesiology and Intensive Care Medicine, Dresden, Germany
[3]CRTD / DFG-Center for Regenerative Therapies Dresden - Cluster of Excellence, Dresden, Germany

Question

Advances in the treatment of nervous system injuries are limited by the delayed and indirect diagnosis of any therapeutic response. The successful regeneration of spinal cord or peripheral nerve injury cannot be monitored until the natural course from reconnection to functional activity is completed. Therefore, there is a clinical need for high-resolution *in vivo* imaging to monitor regeneration of the nervous system. Label-free multiphoton techniques have the ability to close this gap and to advance imaging-based diagnostics. Its potential to image regeneration of the central and peripheral nervous system was studied in the highly regenerative axolotl salamander

Methods

Transection of either the sciatic nerve or the spinal cord were performed. Axolotls were euthanized on day 0, 2, 7, 14, 21, 28, 42 and 100 after injury (n = 10 per group). Cryosections were analyzed with label-free multiphoton microscopy. Second harmonic generation (SHG) showed collagen, two photon excited fluorescence (TPEF) probed endogenous fluorophores and coherent anti-stokes Raman scattering (CARS) tuned on CH_2 vibration visualized lipid-rich structures, i. e. mainly myelinated axons. Conventional immunohistochemistry served as a reference (myelin basic protein (MBP): myelin sheaths, neurofilament (NF): axons, Iba1: microglia). Endogenous signals and immunofluorescence were analyzed in seven regions proximal and distal to the lesion. The neurological status of the animals was assessed after sciatic nerve lesion using a swim analysis to obtain the axolotl sciatic functional index (ASFI).

Results

Multiphoton imaging was able to classify and visualize normal and injured tissue stages in the sciatic nerve. TPEF-positive structures were assigned to macrophages and activated microglia by the comparison with the Iba1 immunohistochemistry and were only found after injury. Upon lesion, their number strongly increased until day 14 (to 140 ± 27 in the lesion center). The relative CARS signal intensity distal to the lesion decreased from day 0 (1.91 ± 0.32) until day 21 (1.45 ± 0.2). It correlated to the diminished MBP signal and represented degeneration of the nerve tissue. Regrowth of axons indicated by NF immunohistochemistry was observed at day 14. Remyelination started at day 21 (identified by CARS and MBP) and corresponded to the time course of functional impairment (ASFI day 0: -1.33 ± 0.17) followed by recovery starting at day 21 (ASFI: -1.03 ± 0.21). After 100 days, the function was completely restored (ASFI: -0.02 ± 0.06); CARS and MBP confirmed intact sciatic nerve morphology with aligned myelin structure and TPEF-positive cells were cleared. The same parameters allowed monitoring the regeneration after spinal cord injury.

Conclusions

Label-free multiphoton imaging allowed following injury induced alterations in the axolotl nervous system, in particular temporal changes of the inflammatory response and the myelination status. High resolution morphochemical information enables to predict successful regeneration before any functional improvement is accessible. Therefore, these findings have the potential to contribute to new strategies for monitoring nervous tissue injuries and improving the effectiveness of therapies.

OP-106 Establishing cell patterns for cell assays

*K. Uhlig[1], R. Wellhausen[1], M. Zeiser[2], T. Wegener[3], T. Hellweg[2], H. Seitz[1], E. Ehrentreich-Foerster[1], C. Duschl[1]

[1]Fraunhofer Institute for Cell Therapy and Immunology, Potsdam, Germany
[2]University of Bielefeld, Physical und Biophysical Chemistry III, Bielefeld, Germany
[3]GeSiM mbH, Grosserkmannsdorf, Germany

Efficient and localised manipulation of cell behaviour is a prerequisite for cell assays aiming to explore novel therapeutic and diagnostic approaches and tools for drug research. In this context, local control over the adhesion of cells to their cultivation substrate plays an important role in investigating and analysing cell response under controlled conditions.

Here, we present two different methods to produce cell patterns for such assays:

The first approach is based on defined positioning of different cell lines using a contact-free technique for dispensing nanolitre volumes. Based on systematic quantitative analyses of cell survival rates, we successfully identified parameter ranges which allow for deposition conditions that are sufficiently gentle to reproducibly maintain cell viability.

The second approach to generate local cell patterns employs lateral deposition of thermoresponsive polymers using an InkJet Printer. Thermoresponsive polymer coatings mediate cell adhesion above a Lower Critical Solution Temperature (LCST) and are cell-repellent below this temperature (Fig. 1). Hence, the local polymer spots under the homogeneously growing cell layer enable local cell detachment after cooling the substrate for 30 minutes at room temperature followed by a gentle rinsing step. We also verified that these cell patterns can be used for *e. g.* cell mobility analysis and co-culture of different cell types.

In a nutshell, we successfully established two alternatives to create cell patterns for cell assay applications. The first of these methods is more suitable for creating co-cultures or single-cell spots, while the second approach lends itself to use in microfluidics and automated cell culture.

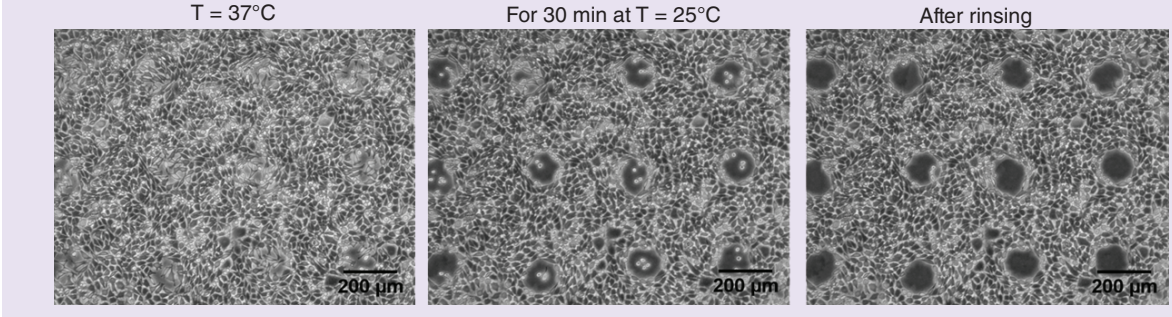


Figure 1. Transmission light microscopy images of L929 mouse fibroblasts growing on a plastics surface, coated with spots of thermoresponsive polymer. Due to temperature reduction across the LCST, the cells on the thermoresponsive polymer detach locally and can be rinsed off.

OP-107 Tracking of autologous transplanted stem cells

*K. von der Haar[1], A. Lavrentieva[1], F. Stahl[1], T. Scheper[1], S. Immenschuh[2], K. Reimers[3], B. Weyand[3], C. Blume[1]

[1]Leibniz Universität Hannover, Institute of Technical Chemistry , Hannover, Germany
[2]Hannover Medical School, Institute for Transplant Immunology, Hannover, Germany
[3]Hannover Medical School, Department of Plastic, Hand and Reconstructive Surgery, Hannover, Germany

Stem cell therapy is a rapidly advancing field in medical research and applications. To reach the full potential of stem cell therapy, it is necessary to examine survival, biodistribution and engraftment of transplanted cells. Additionally, occurrence of genetic aberrations after stem cell transplantation makes it necessary to identify the origin of tumor forming cells. Identification and monitoring of autologous transplanted stem cells is especially challenging, as standard fingerprinting methods cannot be applied. Therefore, autologous transplanted stem cells must be labeled before transplantation. In this project we establish two new approaches to facilitate cell identification: epigenetic barcoding and synthetic DNA labeling as distinctive endogenous cell signatures of substantially manipulated mesenchymal stem cells (MSCs).

Synthetic DNA labeling was first examined using the reporter gene *egfp* on the EGFP-N1 plasmid. Different commercial lipofection kits and electroporation were tested for transient transfection. For electroporation we could reach transfection efficiencies of up to 50% of the cells.

In a first step of epigenetic barcoding, target DNA regions were successfully identified and stem cell DNA was extracted. In order to determine stable methylation patterns, DNA must be sequenced before and after bisulfite treatment, that converts all non-methylated cytosines into uracils. Sequence comparison will lead to methylation patterns. Furthermore, methylation pattern comparison between different cell states (after differentiation / after inflammatory induction) will lead to information about methylation pattern stability. The first steps of methylation pattern analysis were carried out via sequencing before bisulfite conversion. Next, bisulfite converted promoter regions will be sequenced and compared with the original sequence in order to determine the methylation patterns and their stability.

Due to this work we will be able to show two different approaches for labeling systems before autologous stem cell transplantation in order to identify cells after implantation.

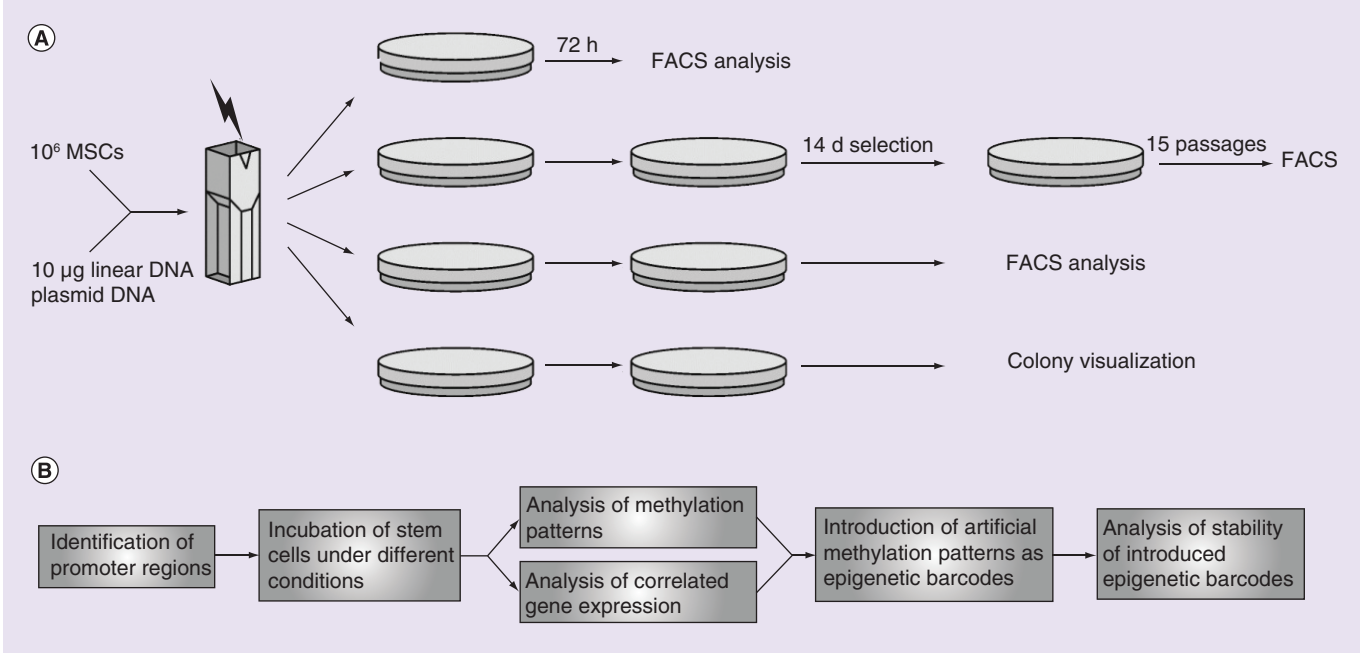


Figure 1.

OP-108 Acceleration of vascularized bone tissue engineering in a large animal model for early clinical applicability

*A. Weigand[1], A. M. Boos[1], J. P. Beier[1], R. Brodbeck[1], T. Bäuerle[2], A. Hess[3], T. Gerber[4], A. Arkudas[1], R. E. Horch[1]

[1]University Hospital of Erlangen, Department of Plastic and Hand Surgery, Erlangen, Germany
[2]University Hospital of Erlangen, Department of Radiology, Preclinical Imaging Platform Erlangen, Erlangen, Germany
[3]Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Experimental and Clinical Pharmacology and Toxicology, Erlangen, Germany
[4]University of Rostock, Institute of Physics, Rostock, Germany

Objective

During the last decades, a range of excellent and promising strategies in Bone Tissue Engineering has been developed. However, in transferring *in vitro* into *in vivo* models or even into clinical settings, various approaches have proven to be unsatisfactory for extensive and complicated bone defects. The remaining major problem is the lack of vascularization. The aim of this study was to enhance vascularization of large scale bone tissue-engineered constructs combining the sheep intrinsic arteriovenous (AV) loop model with additional extrinsic vascularization, while the AV loop maintains its function as main supplying blood vessel for further microsurgical transplantation. In a next step the sheep tibia defect model will pave the way for pre-clinical testing as a basis for early clinical applicability.

Material and Methods

An AV loop was microsurgically created in sheep and implanted within a specially modified perforated titanium chamber together with a primary stable nanostructured bone grafting material, ensuring both intrinsic and extrinsic vascularization. It was compared with the intrinsic vascularization strategy using a closed Teflon® implantation chamber. After different time intervals of up to 18 weeks the vascularization process was monitored by *in vivo* magnetic resonance imaging and post explantation micro-CT analysis. Using (immuno)histological methods, scanning electron microscopy and energy dispersive X-ray spectroscopy bone formation and remodeling of the bone substitute were evaluated and compared between the experimental groups. For proof of concept the sheep tibia defect model was established for first free microsurgical transplantation of an axially vascularized bone substitute in clinically relevant size.

Results

Over time, 3D visualization illustrates the dense vascularization arising from the AV loop. Results could be confirmed by (immuno)histological analyses after explantation. Using the intrinsic/extrinsic vascularization model, the bone substitute was completely interspersed with newly formed tissue after a relatively short time period of 12 weeks. Degradation process of the scaffold and osteoclastic activity were significantly accelerated with the combined intrinsic/extrinsic vascularization after 18 weeks, compared to 12 and 18 weeks of intrinsic vascularization only. Immunohistochemical staining revealed an increase in bone tissue formation over time in both experimental groups. Scanning electron microscopy and energy dispersive X-ray spectroscopy demonstrated successful matrix change from an inorganic to an organic scaffold in vascularized areas. Selective staining of the intrinsic and extrinsic vasculature indicates the connection of extrinsic with intrinsic vessels over time.

Conclusion

In this study the additional extrinsic vascularization of an axially vascularized bone substitute in clinically relevant size promotes tissue ingrowth and remodeling processes of the bone substitute. It was shown that using perforated chambers contributes to faster vascularization and can significantly reduce the time until microvascular transplantation of bone constructs. The sheep tibia defect model creates the basis for early clinical pretesting of this therapeutic concept, offering new opportunities for patients with large scale bone defects in near future.

OP-109 Technical enhancement and cost optimization of sample storage in vapor phase nitrogen

*A. Werner[1], N. Hofmann[2]

[1]ASKION GmbH, Application Team, Gera, Germany
[2]Leibniz University Hannover, Institute for Multiphase Processes, Hannover, Germany

Damage-free freezing, cold handling and long-term storage of biological samples such as cells and tissue are the most important factors of successful establishing cell therapies and tissue engineering. The fact that storage of biological samples at most low temperatures positively influences the sample quality after thawing is strengthened by a steadily increasing number of publications. Not only the storage temperature, but also the handling temperature represents a significant factor influencing the sample quality. Handling within an uninterrupted cooling chain as cold as possible is required to maximize quality.

The second crucial factor in an Biobank is, besides the sample quality, to minimize the storage costs. An effective reduction of costs can be achieved by an increase in capacity of the storage system. However, an increase in capacity by orders of magnitude implies further necessary adaptions of the storage system. This includes the possibility of fully automated management of various sample formats within one storage unit.

It is shown how appropriate technologies are used to significantly increase biomaterial quality after thawing. The use of the best possible freezing regime and a well-matched seeding leads to a 30 % higher cell count after recultivation. The warming of a frozen sample occurs 5 times faster during handling in room temperature compared to handling in a -100°C environment. Cyclical rewarming of samples due to handling processes lowers the cell survival rate by a factor of 20 compared to constant cooling.

Furthermore, a new automated LIN storage device is presented able to hold up to 750,000 vials. It possesses a free choice of different labware operated fully automated in one storage unit. The handling temperature in that device is at -130°C to avoid migratory crystal growth or devitrification in case of vitrified samples. The combination of high capacity and fully automated sample processing leads to a significant drop in both acquisition and running costs.

OP-110 Modulation of the bone marrow niche by breast carcinoma cells

*M. Wobus[1], T. Dittrich[1], A. Dhawan[1], M. Kräter[1], A. Jacobi[2], C. List[1], R. Duryagina[1], J. Guck[2], M. Bornhäuser[1]

[1]Universitätsklinikum Carl Gustav Carus an der TU Dresden, Medizinische Klinik 1, Dresden, Germany
[2]TU Dresden, Biotechnology Center, Dresden, Germany

Background

The bone marrow hematopoietic niche is an anatomic site where hematopoietic stem and progenitor cells (HSPCs) can be sustained. Major cellular components are mesenchymal stromal cells (MSCs). The bone marrow niche can be hijacked by circulating malignant cells not only in patients with hematologic malignancies but also in patients with breast cancer early in the course of disease. These disseminated tumor cells use similar molecules and mechanisms as HSPCs to lodge in the bone marrow and interfere with hematopoiesis.

However, the underlying pathophysiology is incompletely understood. This study aims to identify and characterize potential mechanisms modulating the bone marrow hematopoietic microenvironment by invading breast cancer cells.

Methods

Static cell-cell communication networks, representing the integrated signaling among breast carcinoma cell lines (MCF-7 or MDA-MB-231), MSCs and HSPCs were constructed *in silico* by combining differentially over-expressed genes of the involved cell populations with known ligand-receptor interactions.

For *in vitro* experiments, primary MSCs were isolated from bone marrow aspirates of healthy donors and co-cultured with breast carcinoma cell lines MCF-7 or MDA-MB231 in different direct or indirect systems. The non-malignant epithelial cell line MCF-10A served as a control. After different time points of co-culturing, important MSC characteristics as proliferation, cytokine secretion, differentiation and the mechanical phenotype were investigated.

Results

Breast cancer cells exhibited intensive bi-directional intercellular signaling with MSCs and to a lesser extent with HSPCs. Tumor cell-derived signals were reported to recruit MSCs to sites of breast cancer, activate tumor associated fibroblasts and modify MSC differentiation. Hematopoietic microenvironment-derived signals were predominantly associated with tumor cell attraction and metastatic progression. Potential ligands that protect from metastases were exclusively HSPC-derived.

Using *in vitro* experiments, we detected that both MCF-7 and MDA-MB231 breast carcinoma cell lines or their conditioned medium induced a significantly decreased expression and secretion of the chemokine SDF-1 by MSCs with a consecutive suppression of trans-well migration potential of HSPCs. In contrast, this effect was not observed with MCF-10A non-malignant cells. The SDF-1 down-regulation in MSCs was partly associated with increased TGFb1 signaling since addition of a blocking anti-TGFb1 antibody to MSC/tumor cell cultures completely rescued the SDF-1 secretion. Moreover, the TGFb1 expression levels in MSCs were increased after contact to tumor cell conditioned medium suggesting a modulation of the global bone metabolism. Moreover, osteogenic differentiation of MSCs was inhibited by tumor cells which was accompanied by further reduction by g-secretase inhibitor DAPT which had no effect in Jagged-1 or dnMAML over-expressing cells suggesting an involvement of Notch/Jagged signaling. Moreover, the mechanical phenotype of MSCs was changed after co-culture with breast carcinoma cells.

Conclusions

In conclusion, we propose a modulation of MSCs by breast cancer cells by different mechanisms and the mechanical phenotype. These indirect changes in the bone marrow niche upon tumor cell invasion might increase the vulnerability for bone metastasis in breast cancer patients but also provide potential therapeutic targets.

OP-111 Novel 3D textile scaffolds for *in situ* bone tissue engineering using silk fibers functionalized with hPDGF and osteoconductive ceramic materials.

*M. Wöltje[1], R. Brünler[1], C. Adamzyk[2], D. Aibibu[1], M. Böbel[3], G. Müller-Newen[4], S. Ernst[2], S. Neuss[2,5], C. Cherif[1]

[1]Institute of Textile Machinery and High Performance Material Technology / TU Dresden, Dresden, Germany
[2]Institute of Pathology / RWTH Aachen, Aachen, Germany
[3]Spintec Engineering GmbH, Aachen, Germany
[4]Institute of Biochemistry and Molecular Biology / RWTH Aachen, Aachen, Germany
[5]Helmholtz Institute for Biomedical Engineering / RWTH Aachen, Aachen, Germany

Objectives

Regeneration of damaged or diseased tissues and organs by tissue engineering represents a promising tool in future therapies. In general, this includes three steps: 1) design of a three dimensional scaffold ideally mimicking the natural environment of damaged tissue, 2) selection of an appropriate cell type which could be readily isolated, expanded, and differentiated into cells of the target tissue, and 3) implantation of the *in vitro* generated copy of the target tissue to substitute function *in vivo*. Even though, this strategy scientifically sounds coherent, transfer into clinics bears many obstacles in terms of regulatory requirements. Those cell or tissue containing constructs that have been modified to be used to repair, regenerate or replace human tissues are classified as Advanced-therapy medicinal products (ATMPs). Thus, ATMPs have to be evaluated for safety and efficacy like any other conventional medicines which means long timescales and high costs until such an experimental product might reach the clinics. Therefore, the focus of this work was to develop a suitable scaffold design to enable cellular ingrowth and tissue reconstruction *in situ*. This includes generation of highly porous textile three-dimensional constructs using silk fibers functionalized with human platelet derived growth factor (hPDGF) and osteoconductive ceramic materials. Functionality in terms of osteogenic differentiation and vascularization using co-cultures of mesenchymal stem cells (MSC) and human umbilical vein endothelial cells (HUVEC) was investigated.

Material and Methods

Transgenic silkworms were generated to functionalize silk proteins with human platelet derived growth factor (hPDGF). These genetically modified silk proteins were isolated and spun into fibers presenting hPDGF. In addition, silk fibers containing osteoconductive ceramic materials (hydroxyapatite, HA and beta-tri-calcium-phosphate, ß-TCP) were spun. Applying short fiber based Net Shape Nonwoven (NSN) technology, interconnected porous 3D constructs were manufactured from hPDGF and ceramic modified silk fibers. Scaffolds were characterized in terms of porosity, compressive strength, and cyclic load. For evaluation of cytocompatibility according to ISO 10993-5, viability of cells was monitored using live/dead staining and CellTiter Blue assay. FACS was used for characterization of MSC and HUVEC. Osteogenic differentiation was determined by *realtime* PCR. Spatial distribution of cells and vascularization were visualized by laser scanning microscopy.

Results and Conclusion

Silk fiber based 3D-hybrid scaffolds showed cytocompatibility and a tendency of increased proliferation of HUVEC and co-cultured MSC was observed on PDGF-functionalized silk. MSC cultured on HA- and ß-TCP/HA-functionalized silk scaffolds expressed higher levels of osteogenic marker genes (Runx2, ALP). Capillary-like structures were observed in all scaffold types, whereas higher matrix density and more capillary-like structures were determined for HA- and PDGF-containing scaffold types. In conclusion, 3D interconnected porous textile scaffolds were proved as promising biomaterials for bone regeneration *in situ*.

OP-114 Functional features and therapeutic availability of cultured adult multipotent mesenchymal stromal cells of different tissue origin

*D. Zubov[1,2], R. Vasyliev[1,2], A. Rodnichenko[1,2], A. Zlatska[1,2], O. Gubar[2], S. Novikova[1]

[1]State Institute of Genetic and Regenerative Medicine, National Academy of Medical Sciences of Ukraine, Cell & Tissue Technologies Unit, Kiev, Ukraine
[2]Medical company ilaya®, Biotechnology laboratory ilaya.regeneration, Kiev, Ukraine

Objective

The multipotent mesenchymal stromal/stem cells (MSCs) of different tissue origin when cultured under specific conditions reveal a whole range of morphological and functional distinctions. It is also known that for recovery of any tissue it is preferably to use the histotypical cells characteristic to the tissue to be regenerated. For example, for cartilage repair it is preferable to use chondrocytes, for bone - periosteum progenitor cells (PPCs), and for endothelial dysfunction correction and tissue ischemic damage - endothelial progenitor cells (EPCs). Moreover, there exists a positional memory of cells originated from different areas of the human body, e.g., the dermal fibroblasts and keratinocytes from different skin areas differs not only by specific HOX code but both intracellular and secreted proteins profiles [1,2]. The aim of our study was to evaluate some of the functional characteristics of adult MSCs and their derivative cell types from a variety of tissue sources in the culture altogether with the assessment of their availability for regenerative medicine use.

Material and methods

Cell culture of human adult MSCs originated from: bone marrow, adipose tissue, endometrial stroma, Hoffa's fat pad, placenta, synovial membrane, as well as derma, periosteum, hyaline cartilage. Flow cytometry; differentiation into the orthodoxal trilineage directions; ELISA; bright field and fluorescence microscopy; statistical analysis.

Results and conclusions

There were revealed functional distinctions of cultured adult MSCs of different tissue origin under various culture conditions (21% O_2 and 5% O_2 content) and with use of different growth media such as supplemented with conventional heterologous growth substances (FBS) and homologous products (human pooled serum and platelet lysate) and synthetic commercial medium: immunophenotype, trilineage differentiation potential, growth kinetics, pro- and anti-inflammatory cytokines production level under influence of exogenous cytokines supplementation, chemical and physical stimulation *in vitro* (TNF-α, IL-1β, IL-10, IL-1RA). As a practical result of a study it was recommended to seed an original 3D Tissue-engineered bone equivalent with a mix of autologous cultured bone-marrow derived MSCs and PPCs (3:1) for use in bone defect reconstruction. Cultured adult MSCs of different tissue origin correspond to the minimal criteria defined by the International Society for Cellular Therapy in a position statement [3].

References

1 Chang HY, Chi J-T, Dudoit S *et al*. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc. Natl Acad. Sci. USA* 99(20), 12877–12882 (2002).

2 Rinn JL, Bondre C, Gladstone HB, Brown PO, Chang HY. Anatomic demarcation by positional variation in fibroblast gene expression programs. *PLoS Genet.* 2(7): e119 (2006).

3 Dominici M, Blanc KL, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4), 315–317 (2006).