Abstracts of the 2nd Microphysiological Systems World Summit, Berlin, 2023

Volume 11, No. 1
ISSN 2194-0479
doi:10.58847/ap.2301 (2023)

ALTEX Proceedings

Springer Spektrum

Dear colleagues, collaborators, and friends,

In the last decade, the development of MPS (microphysiological systems) has surpassed all expectations. Originally limited to basic and clinical research, these technologies have rapidly progressed to industrial applications in drug discovery and even the production of data for regulatory decisions. This breakthrough has sparked the creation of numerous new enterprises worldwide, eager to capitalize on the technology's potential. MPS can now emulate genuine human biology under experimental conditions, potentially reducing the need for animal testing.

"*MPS can be considered the flagship of animal-free methods. Non-regulated fields, like basic medical research and drug discovery, have already fully embarked. Now, many opportunities are arising for integration of regulatory applications in the large fleet of MPS outputs*." – Marcel Leist – University of Konstanz/CAAT-Europe (Germany)

Following the success of the first MPS World Summit in New Orleans, where the International MPS Society (iMPSS) was inaugurated, the 2nd MPS World Summit is taking place in the heart of Europe.

"*By combining forces between the iMPSS and the European Organ-on-Chip Society (EUROoCS), we are able to revolutionize the field of biomedical research and accelerate the development of human-relevant approaches to understanding health and disease. Together we ensure a comprehensive exploration of the latest advancements in microphysiological systems and gain deeper insights into the development and implementation of OoC platforms, their integration with cutting-edge technologies, and the exciting possibilities they offer in addressing key biomedical challenges*." – Peter Loskill – University of Tübingen (Germany) / EUROoCS

Under the motto "Emulating human biology for patients' benefit and a safer environment in the 21st century and beyond!" the summit is bringing to Berlin (Germany) the academic research community, medical centers, the pharmaceutical, cosmetics, chemical and food industries, regulatory agencies, health foundations, charities, patients' associations, and policymakers.

"*Berlin is a vibrant city with an eventful history, especially in the last 150 years. In the last 15 years, it has also become a hotspot for the invention, development, and use of MPS tools in medical research and drug development. Berlin hosted the two international CAAT stakeholder workshops on biology-inspired MPS in 2015 and 2019*." – Uwe Marx – TissUse GmbH / Technische Universität (Germany)

This year, the MPS World Summit has exceeded all expectations, with 750 abstract submissions, more than 160 speakers in more than 30 sessions, over 560 posters, 75 sponsors, and approaching 1200 registrations at the time of writing from all parts of the world, showing the enormous interest of the scientific community.

"*It takes a village to raise a child, and our MPS society is becoming a large town of highly dedicated researchers working on MPS research and development! It is exciting to see the growth of interest and rise in implementation of these technologies across the globe – a truly international effort!*" – Lena Smirnova – Johns Hopkins University, Center for Alternatives to Animal Testing (USA) / iMPSS

Marcel Leist
(MPS WS 2023 host)

Uwe Marx
(MPS WS 2023 host)

Peter Loskill
(MPS WS 2023 host)

Lena Smirnova
(iMPSS, chair of the program committee)

James Hickman
(iMPSS, chair of the fundraising committee)

Jan Lichtenberg
(iMPSS)

Riccardo Barrile
(iMPSS, chair of the education program/workshop)

Thomas Hartung
(NCATS grant PI)

Anwyn Statnick
(meetings organizer)

Camila Sgrignoli Januario
(meetings organizer)

The conference will not only be about science but also about the people developing, applying, and believing in the strength of the MPS technology. For this, several networking activities have been planned: starting with the welcome reception and moving to the "Macro Party" at the Spree River. The MPS World Summit "matchmaking" will facilitate continuous connections during the event.

We welcome all of you to Berlin to connect, exchange, and learn about MPS. Enjoy the science and the city!

Marcel Leist, Uwe Marx, Peter Loskill (WS 2023 hosts) and
Lena Smirnova (iMPSS board president)

The MPS World Summit is supported by NCATS through a U13 TR000395 conference award and sponsors.

Dear MPS World Summit participants,

ALTEX Proceedings is honored to publish the Abstract Book of the 2nd Microphysiological Systems World Summit in Berlin, Germany.

After the great success of the 1st MPS World Summit held in hybrid format in New Orleans in 2022, the 2nd MPS WS in Berlin has attracted even more participants, who are eager to meet in person, exchange knowledge, and expand their networks.

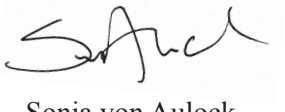
This Abstract Book contains more than 750 abstracts for invited talks, oral presentations, and posters sorted by submission ID number. They represent the work of more than 3000 contributing authors from 45 countries on all six continents. The talks are arranged into four tracks: MPS development: bioengineering models and readouts; MPS for industrial and regulatory application: Standardization, QA, parallelization and automation; MPS for disease modelling, safety testing and basic research; and MPS highlights across disciplines. Each track comprises eight sessions. Please consult the program for details.

We are grateful to the Doerenkamp-Zbinden Foundation, Kreuzlingen, Switzerland for funding the production of this Abstract Book, which is the formal record of the Summit. Many thanks to Lena Smirnova and Anwyn Statnick from the Center for Alternatives to Animal Testing at Johns Hopkins University, Baltimore, MD, USA for a smooth cooperation.

We wish all participants of the 2nd MPS World Summit a memorable meeting that inspires new projects and fruitful collaborations towards human-relevant research without the use of animals to improve the health and safety of humans with microphysiological systems.

We can already look forward to the 3rd MPS World Summit to be held in Seattle, WA, USA in 2024.

With best wishes,



Sonja von Aulock
Editor in chief, ALTEX & ALTEX Proceedings

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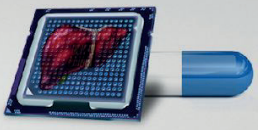


Quris is an artificial intelligence innovator with goal to disrupt the drug development process. Our Bio-AI platform better predicts which drug candidates will safely work in humans, avoiding the tremendous costs of failed clinical trials and animal testing. Quris is already working with leading pharma companies to evaluate the safety profile of pre-clinical and clinical assets.

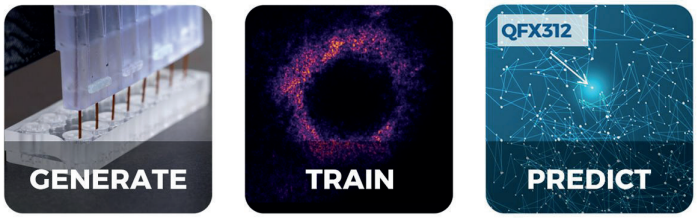
Unmet Need: Drug safety is a major unaddressed problem. A staggering 92% of all drugs fail in clinical trials, despite 'successfully' passing animal testing, costing pharma companies over $53B each year. There is currently no company or solution that addresses this challenge: predicting which drug candidate will be safe in the human body, and for whom.

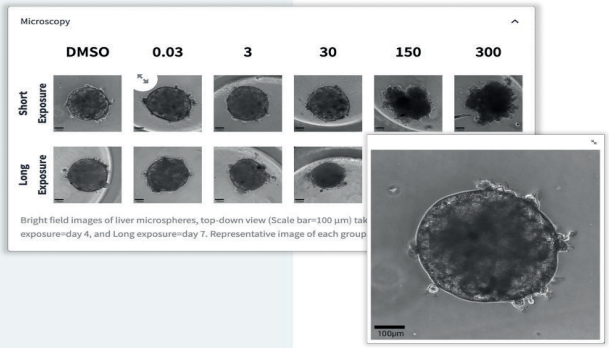
Bio-AI Platform: Quris uniquely combines the power of cutting-edge ML together with patients-on-chip technology, to better predict drug safety. How does it work? While the science and technology are complex, its essence is a simple, three-tiered process:

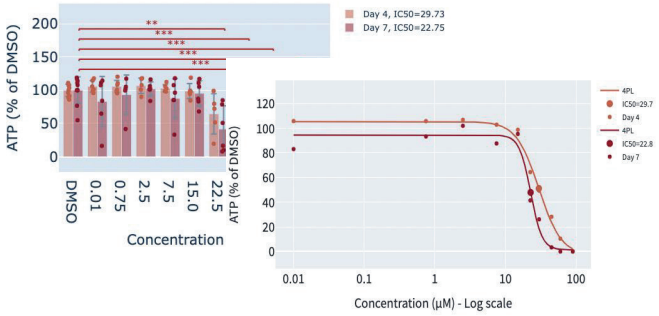
Generate millions of interactions between known drugs (safe drugs and toxic ones) and patients-on-chip (miniaturized interconnected human organs on a chip).

Train the AI model, based on the proprietary multi modality labeled data. Including microscopy images and proprietary nano-sensing.

Predict whether a new drug candidate will be safe to the human body, and for whom







Stellar Team: Based in Boston and Tel-Aviv, Quris is led by a team of track-record pioneers in the fields of machine-learning, statistics, biology, software, genomics, engineering, and med-tech – all with a strong track record of success, including Moderna's co-founder Langer, Nobel laureate Ciechanover, and former Pfizer CEO McKinnell. The founders authored 48 patents, led two FDA approved products, and multiple successful Life-Sci exits (M&A, NASDAQ IPO).

"Quris is going to have a far greater impact on the pharmaceutical industry and world-health than anybody realizes."

Henry McKinnell, former Pfizer CEO

Abstracts

4

h-VIOS: A novel human organ-on-a-chip platform using vascularized biomaterials

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Organs-on-chips and tissue engineering have revolutionized the way we think about modeling human physiology and pathology *in vitro*. However, a significant challenge has posed a limitation in the advancement of these models: the ability to introduce vasculature into tissues, which is crucial for cell survival and function. 3D Systems technology enables the creation tissues out of materials that mimic the extracellular matrix, containing a vascular network that provides nutrient delivery and waste removal. Using 3D Systems 3D printing and bioprinting technologies, Systemic Bio has invented the h-VIOS platform, which enables scalable production of vascularized hydrogels compatible with a variety of cell types, from healthy and diseased cell lines to stem cells and human primary cells, which can be used for drug testing. In this presentation, we will go over the h-VIOS system and how it has been used to model different organ and disease functions *in vitro*.

Presentation: Poster

5

Angiogenesis-driven extracellular matrix remodeling of 3D bioprinted vascular networks for *in vitro* therapeutic testing

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Angiogenesis plays a pivotal role in development and tissue growth, as well as in pathological conditions such as cancer. Being able to understand the basic mechanisms involved in the vascularization of tissues and angiogenic network formation provides a window to advance the development of *in vitro* tissue models and enhance tissue engineering applications. In this study, we leveraged a novel microfluidic-based three dimensional (3D) bioprinting technology and alginate-collagen type I (AGC) bioink, to develop a 3D bioprinting strategy to enable the biofabrication of complex angiogenic networks within the 3D structure. These networks were comprised of simian vacuolating virus 40 (SV40) transformed adult rat brain endothelial cell (SV-ARBEC)-laden hydrogel rings. With mechanical properties relevant for vascular tissue engineering applications, these bioprinted constructs formed spontaneous vascular networks, reminiscent of anisotropic tissue-like structures, while retaining high cellular viability. The vascular network formation was accompanied by extracellular matrix (ECM) remodeling, confirming sequential SV-ARBEC mediated collagen type I fiber deposition and reorganization. Treatment with broad spectrum matrix metalloproteinase (MMP) inhibitor supressed SV-ARBEC angiogenic sprouting, highlighting requirements of ECM remodeling in angiogenic network formation. This novel 3D microfluidic bioprinting technology and biocompatible AGC hydrogel fiber rings supported robust SV-ARBEC angiogenesis and corresponding ECM remodeling, allowing us to present a strategy suitable to advancing applications in vascular research and supporting the further development of disease models, novel testing beds for drug discovery and tissue engineering applications.

Presentation: Poster

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Fun with NAMs

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Education brings neither glory to the scientists nor a better h-index. Nevertheless, it is useful for multiple reasons such as knowledge sharing, capacity building and creation of an adequate ecosystem. Overall, one can admit that the education and training about 3Rs at university level has the merit to exist even if it could be possibly better advertised and communicated. The JRC launched a mapping exercise on this matter in 2018 but as far as the authors are concerned the results of the study were not published [1]. A category of individuals that is rarely targeted properly is the general public as well as teaching at primary and secondary school. JRC took care of the latter by providing learning scenarios to empower the teachers [2]. Moreover, organising open days as well as participating in science festivals are great venues for reaching out the general public. Still, there is space for creativity by providing other formats. At Altertox, two new concepts and formats are expected to complement the current "arsenal" of tools available. The first one is an edutainment game meant to open a conversation about NAMs (New Approach Methodologies) and validation process in a fun and convivial environment. "TATABOX" (Towards Alternatives To Animal testing) tiles are not meant to be exhaustive in terms of content as well as persona but rather a starting point for discussion with concrete items within a team on the process towards regulatory acceptance. The second one is a quiz game for youngsters (from 9+, "Little genius") meant to raise public awareness about NAMs, laboratory animals, life sciences, legislation in Europe and NAMs job. Questions for quiz were adapted to general public using true/false, multiple choices, open ended questions. Hopefully, these two new formats will provide supplementary ways for the scientific community to exchange at national level.

References

[1] https://joint-research-centre.ec.europa.eu/jrc-news/education-and-training-3rs-2018-02-27_en
[2] Introducing the Three Rs into secondary schools, universities. JRC123343. https://publications.jrc.ec.europa.eu

Presentation: Poster

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Altering cell fate transitions of human iPSCs during hepatic lineage specification

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Induced pluripotent stem cells (iPSCs) are a valuable resource for interrogating the molecular mechanisms underpinning lineage specification. Understanding interactions between signaling pathways is central to the differentiation of iPSC towards a hepatic fate and contributes to their physiological relevance [1]. The Hippo signaling pathway regulates dynamic cell fates in the liver [2] with its downstream effector, Yes-associated protein 1 (YAP), playing an essential role in lineage specification as a transcriptional coactivator that is associated with cis-acting DNA regulatory elements-enhancers [3]. The functional significance of YAP chromatin occupancy in iPSCs, and its subsequent impact on hepatic specification, are not fully understood.

Human iPSCs were exposed to bidirectional toggling of YAP subcellular localization with small molecules and differentiated using growth factors for 30 days [1]. Differentiated cells were characterized for relative gene expression, albumin secretion, and CYP3A4 enzymatic activity (P450-Glo™ Assays and LC-MS/MS). Epigenetic markers associated with active enhancer elements (H3K4me1, H3K-27ac), repressive chromatin (H3K27me3) and YAP chromatin occupancy were assessed using Cleavage Under Targets & Release Using Nuclease (CUT&RUN).

Transiently altering YAP subcellular localization, by increasing nuclear localization prior to onset of differentiation, altered functional and transcriptional phenotypes. CYP3A4 enzymatic activity, measured between day 25-30, was improved 4-fold. This was accompanied by temporal differences in the relative gene expression of key lineage markers across definitive endoderm, ventral posterior foregut, and maturation stages of HLC differentiation. Altered YAP occupancy and chromatin dynamics in iPSCs accelerated the differentiation kinetics of iPSC towards a hepatic fate. YAP regulates cell fate in the liver and could be a favorable target in improving the acquisition of hepatic fate during iPSC differentiation.

References

[1] Raggi et al. (2022). Leveraging interacting signaling pathways to robustly improve the quality and yield of human pluripotent stem cell-derived hepatoblasts and hepatocytes. *Stem Cell Rep 17*, 584.
[2] Nguyen-Lefebvre et al. (2021). The hippo pathway: A master regulator of liver metabolism, regeneration, and disease. *FASEB J 35*, e21570.
[3] Sun et al. (2020). Hippo-YAP signaling controls lineage differentiation of mouse embryonic stem cells through modulating the formation of super-enhancers. *Nucleic Acids Res 48*, 7182.

Presentation: Poster

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Exposing the pathways in embryo morphogenesis by phenotypic screening of embryo models

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Embryogenesis is an integrative process supported by dynamic loops of cellular interactions. Stem cell-based embryo models open exciting new opportunities to study these processes *in vitro*. Here, we create a partial mouse embryo model to elucidate the principles of epiblast (Epi) and extraembryonic endoderm (XEn) co-development. We chemically trigger naive mouse embryonic stem cells to form a blastocyst-stage niche of Epi/XEn-like (3D, hydrogel-free, serum-free). Once established, these two lineages conjointly recapitulate successive morphogenetic events of the peri-implantation embryo, including cell sorting, cell polarization, pluripotency exit, pro-amniotic cavity-like formation and transcriptomic channeling. This autonomous progression occurs through a pendulum of reciprocal inductions propagating over time. Similarly, in blastoids, co-inducing the formation of Epi- and PrE-like cells supports their developmental progression. We then translate this embryo model to a thin polymer film-based thermoformed microwell platform to enable fluorescence imaging-based feature detection and use machine learning to classify morphogenesis, including embryo model heterogeneity present in the system. Through screening with a soluble compound library, we delineate the molecular pathways involved in embryonic progression. We conclude that combining embryo models with advanced culture platforms opens the door to large-scale screening assays with impact on embryology, embryo-toxicity, drug development, and reproductive medicine.

Presentation: Oral

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A human multi-organ and dynamic *in vitro* model for simultaneous and more predictive toxo-efficacy assays

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Introduction: In the oncology field, the need to address the poor success rate of clinical trials is becoming more and more evident. Therefore, the development of more predictive systems involving dynamic cues and organ-organ interactions is fundamental for evaluating the pharmacodynamic of novel anti-cancer treatments.

A novel multi-compartmental and flexible organ-on-chip (OOC) platform has been adopted to fluidically connect a 3D ovarian cancer tissue to a hepatic cellular model, and to resemble the systemic cisplatin administration for investigating drug efficacy and contemporarily measuring potential hepatotoxic effects in a physiological context.

Material and methods: Human liver HepG2 and ovarian cancer SKOV-3 cell lines were cultured both in 2D and in 3D, within an alginate-based hydrogel [1]. A co-culture model was implemented by cultivating SKOV-3 within porous permeable inserts accommodated within the 24-well plates where HepG2 were plated on the bottom.

A compartmental fluidic device [2] was adopted to assess simultaneously the on-target and off-target cytotoxic activity of cisplatin under dynamic conditions.

For the dynamic multi-organ condition, two independent OOC chambers hosting liver and ovarian cancer models were fluidically connected to each other by the external fluidic circuit, reproducing the *in vivo*-like tissues arrangement and inter-connections.

Results: A linear decay of both Skov-3 ovarian cancer and Hep-G2 liver cells viability was observed with increasing cisplatin concentration after 48 h of treatment. Furthermore, the 3D ovarian cancer model showed a higher drug resistance than the 2D model, when cultured in static condition. Most importantly, the combinatory experimental approach of 3D culture, fluid dynamic condition and multi-organ connection displayed the most predictive toxicity and efficacy outcomes compared to the clinical data.

Conclusion: These results open new challenging opportunities for OOC based approaches in preclinical drug discovery, in the same direction of the 3R based regulatory changing.

References

[1] Marrella, A. et al. (2021). 3D fluid-dynamic ovarian cancer model resembling systemic drug administration for efficacy assay. *ALTEX 38*, 82-94. doi:10.14573/altex.2003131

[2] Marzagalli, M. et al. (2022). A multi-organ-on-chip to recapitulate the infiltration and the cytotoxic activity of circulating NK cells in 3D matrix-based tumor model. *Front Bioeng Biotechnol*, 10, 945149.

Presentation: Poster

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3D vessel-gut-on-chip platform investigating interactions between immunological system and tumoral tissue remodelling and angiogenesis

Karol Kugiejko[1,2], Mattia Ballerini[1,2], Luigi Nezi[2] and Marco Rasponi[1]

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Researchers are increasingly interested in the balance and makeup of bacteria in the intestine, which can change over time. Bacteria play an important role in the response to cancer immunotherapy, but traditional cell cultures and animal models have limitations in studying this process.

Gut-on-Chip (GoC) devices are promising *in vitro* model for studying the complex interactions between the gut microbiome and the intestinal lining. In this study, we present a 3D Vessel-Gut-on-Chip (VGoC) platform that investigates the interactions between the immune system and tumoral tissue. The platform is made of polydimethylsiloxane (PDMS) and is shaped like a blood vessel and is populated with cells that mimic the functions of blood microvasculature (HMEC-1), cells from the intestinal lining, and immune cells such as PBMCs, T cells, B cells, and macrophages. The platform can be mechanically stimulated to replicate the peristaltic movements and forces experienced by blood vessels and intestinal tissues *in vivo*.

By studying the interactions between the immune system and tumoral tissue on this platform, we aim to gain a deeper understanding of the immune system's role in tumoral tissue remodelling and angiogenesis. This knowledge may aid in identifying novel therapeutic targets for treating cancer and other diseases involving abnormal tissue growth and blood vessel formation. In addition, the VGoC platform can be used to evaluate the effects of pharmacological agents or other interventions on the immune system and tumoral tissue.

The 3D VGoC platform represents a valuable tool for studying the complex interactions between the immune system and tumoral tissue remodelling and angiogenesis. Its ability to mimic the intricate multicellular architectures and niches of the gut and its capacity to physically stimulate cells allows for a more realistic and informative *in vitro* model. The findings from this platform may facilitate the development of novel therapeutic strategies for cancer and other diseases involving abnormal tissue growth and blood vessel formation.

Presentation: Poster

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Using microphysiological systems to develop treatments for joint inflammation and associated cartilage loss – a pilot study

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Osteoarthritis (OA) is a painful and disabling joint disease that affects millions of people worldwide. The lack of clinically relevant models limits our ability to predict therapeutic outcomes prior to clinical trials where most drugs fail. Therefore, there is a need for a model that accurately recapitulates the whole joint disease nature of OA in humans. The emerging microphysiological systems provide a new opportunity. We recently established a miniature knee joint system, known as the miniJoint, in which human bone marrow-derived mesenchymal stem cells (hBMSCs) were used to create an osteochondral complex, synovial-like fibrous tissue, and adipose tissue analogs. In this study, we explored the potential of miniJoint in developing novel treatments for OA by testing the hypothesis that co-treatment with anti-inflammation and chondroinducing agents can suppress joint inflammation and associated cartilage degradation. Specifically, we created a "synovitis"-relevant OA model in the miniJoint by treating synovial-like tissues with interleukin-1β, and then a combined treatment of oligodeoxynucleotides (ODNs) suppressing the nuclear factor kappa beta (NF-κB) genetic pathway and bone morphogenic protein- 7 (BMP-7) was introduced. Our findings indicated the combined treatment with BMP-7 and ODN reduced inflammation in the synovial-like fibrous tissue and showed an increase in glycosaminoglycan formation in the cartilage portion of the osteochondral complex. This study for the first time indicated the potential of miniJoint in the development of disease-modifying OA drugs. The therapeutic efficacy of co-treatment of BMP-7 and NF-κB ODNs can be further validated in future clinical studies.

Presentation: Oral

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Utilizing microphysiological systems to model major hallmarks of amyloid β-driven neuronal aging and assess drug applications in Alzheimer's disease

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Organ-on-a-chip is receiving considerable attention due to its versatility in constructing either single or interconnected organoids for noninvasive measurements of cellular function and modeling human diseases with clinical significance [1-3]. The amyloid hypothesis places the accumulation and deposition of fibrillar amyloid-β (Aβ) plaques at the center stage of neurodegeneration and cognitive decline, culminating in dementia [4]. Since AD is an age-risk factor [5], we hypothesized that organoid models must include signatures of neuronal aging. Thus, this work presents an *in vitro* pro-gerontic organoid model comprised of human-induced pluripotent stem cell (hiPSC)-derived cortical neurons cultured on titanium nitride-microelectrode arrays in serum-free defined conditions, highlighting signatures of Aβ-driven aging and revealing intrinsic pathomechanisms of cortical degeneration. In parallel, this defined model measures the electrical activity of neurons in response to Aβ and therapeutic molecules in real-time. Aβ controls Tau phosphorylation through NMDA Receptor- and Src/Fyn-mediated signaling axis, impairing long-term potentiation (LTP) of neurons. Aβ drives neuronal senescence, induces the aberrant production of reactive oxygen species (ROS), and impairs mitochondrial potential. Aging cortical neurons secrete pro-inflammatory factors detected in brain, plasma, and cerebral spinal fluid of AD patients, in which drugs modulate the inflammasome. This functional pro-gerontic model could be utilized in drug screening programs to discover novel therapies to treat human neurological disorders.

References

[1] Ingber, D. E. (2022). Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat Rev Genet 23*, 467-491.

[2] Autar, K. et al. (2022). A functional hiPSC-cortical neuron differentiation and maturation model and its application to neurological disorders. *Stem Cell Rep 17*, 96-109.

[3] Caneus, J. et al. (2020). A human induced pluripotent stem cell-derived cortical neuron human-on-a chip system to study Aβ42 and tau-induced pathophysiological effects on long-term potentiation. *Alzheimers Dement (N Y) 6*, e12029.

[4] Tanzi, R. E. (2005). The synaptic Aβ hypothesis of Alzheimer disease. *Nat Neurosci 8*, 977-979.

[5] Sala Frigerio, C. et al. (2019). The major risk factors for Alzheimer's disease: Age, sex, and genes modulate the microglia response to Aβ Plaques. *Cell Rep 27*, 1293-1306 e6.

Presentation: Poster

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Eye damage reversibility in an *in vitro* model of bovine cornea to replace the Draize test completely

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One of the requirements for the registration of chemicals is to provide evidence about their potential eye damage. The Draize test performed in rabbits allows the products to be classified into four categories, considering both the severity of the lesions produced in the animal's eye as well as its healing time. The available alternative methods to this live animal test do not allow documenting the damage reversibility, nor the time necessary for such reversibility to occur, as required by the UN GHS classifications.

Our proposal is to complement the *in vitro* model that uses the bovine cornea as a substrate to predict whether a substance is irritating or non-irritating (BCOP), with a strategy that allows predicting if the observed irritation is reversible and the time it takes to revert.

Limbal stem cells are known to play an important repairing role in corneal injury; therefore, we isolated these cells from bovine cornea and used them to evaluate the cell sensitivity to reference products. Also, a wound healing assay was performed to study whether these products differentially affect the replication and migration capacity of the cells. Furthermore, a tissue explant and an organotypic cornea culture model were implemented to study if the chemical exposure alters cell's replication, migration, and overall wound healing differentially.

A combination of the approaches used have been proven effective to detect the four categories of GHS reference products. This project complements the BCOP *in vitro* model with a strategy that aims to predict if the observed irritation is reversible which is necessary to finally replace the Draize test completely.

Presentation: Poster

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The use of tissue chips for precision medicine studies

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Microphysiological systems, also known as tissue chips, are a disruptive technology with the potential to transform the drug development process. Since 2012 the NIH Tissue Chip Program has supported research on the use of tissue chips to predict drug safety, toxicity, and efficacy. The newest initiative launched in 2021, "Clinical-Trials-on-a-Chip" (CToC), aims to develop tissue chip technology to inform clinical trial design, planning, and implementation in precision medicine. Within the initiative, tissue chips are being used to include patient diversity, help establish patient recruitment criteria, stratify patients based on response or lack of response to candidate therapeutics, and develop clinically relevant biomarkers and reliable clinical trial endpoints. Funded projects will directly compare tissue chip data to clinical studies, i.e., prospective, retrospective, and ongoing clinical trials. Research conducted within this initiative will help us better understand if tissue chips could be employed as effective patient surrogates within the clinical trials process, perhaps shortening the times it takes to complete clinical trials and thus accelerate drug development. This review will discuss the current state of tissue chip research being conducted within the initiative, as well as what the future holds for tissue chips and their potential use within the precision medicine space.

Presentation: Oral

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Development of a brain on a chip to evaluate compounds for the treatment of Parkinson's disease

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Neurodegenerative diseases are the second largest cause of death and the leading cause of disability worldwide, with an increasing incidence due to the progressive aging of the global population [1,2]. Development of treatments for diseases, such as Parkinson's, is challenging, time consuming and expensive, as the exact disease pathophysiology is yet to be fully understood. This problem is coupled with the difficulty in recapitulating human disease using conventional static or *in vivo* animal models which lack complexity or are subjected to species differences respectively. Furthermore, there is a scarcity and inaccessibility of human samples, so there is a requirement for more physiologically relevant complex *in vitro* models, to better model the interfaces and interactions that occur in human disease, such as the difficulty of penetrating the blood brain barrier. An example of such models is the emerging organ on a chip technology, which introduces relevant *in vivo* forces (shear stress) and environments (gradient formation, nutrient supply, and waste removal) via microfluidics. These devices also enable the inclusion of different cell types in dedicated chambers to study barrier formation, cellular cross talk and can incorporate electrodes for real time assessment of barrier integrity and neuronal activity. Commercialised Organ on a Chip technology was used to generate a brain on a chip to model interactions with blood brain barrier and dopaminergic neurons and compared to conventional static cultures, with the aim of validating and implementing the model into Parkinson's disease drug discovery and development pipelines.

References

[1] Holloway, P. M. et al. (2021). Advances in microfluidic in vitro systems for neurological disease modeling. *J Neurosci Res 99*, 1276-1307. doi:10.1002/jnr.24794

[2] Deuschl, G. et al. (2020). The burden of neurological diseases in Europe : An analysis for the global burden of disease study 2017. *Lancet Public Health 5*, e551-e567. doi:10.1016/S2468-2667(20)30190-0

Presentation: Poster

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In vitro intestine models with *in vivo* like barrier properties for multi-OoC models

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Background: The human small intestine is one of our major body barriers. In addition to food uptake, it separates our internal organs from harmful external factors. Therefore, it is most important that small intestine models optimally mimic the permeability barrier when considering incorporation into multi-organ-on-chip models (OoC) and systemic biodistribution studies. Existing models are not representative of the small intestine as they exhibit abnormally high transepithelial-electrical-resistance (TEER) and low permeability.

Aim: The aim of this study was to develop a human small intestine model that recapitulates the native barrier properties and that can be incorporated into multi-OoC models.

Methods: We compared an epithelial model (epithelial cells seeded directly on a transwell) to a full thickness model (epithelial cells seeded on a fibroblast populated hydrogel). For each model, the intestinal epithelial layer consisted of either Caco2-HT29 co-culture or epithelial cells derived from primary human duodenum organoids. Fibroblasts were isolated from primary human duodenum. Furthermore, fibroblast secretome was applied to epithelium to investigate effects of paracrine factors. Readouts were histology, protein expression (immunohistochemistry), TEER, zonulin expression (positively correlating with permeability) and chemokine secretion (ELISA).

Results: The epithelial cells in all models expressed specific intestinal epithelial markers VIL, MUC2, LYZ, CHGA. The epithelial model, in the absence of fibroblasts, expressed abnormally high TEER and low zonulin secretion. Notably, the presence of living fibroblasts in the lamina propria hydrogel, or the secretome of fibroblasts, remarkably reduced TEER levels to those of native small intestine with a corresponding increase in zonulin secretion. Furthermore, fibroblast-epithelium crosstalk was found to be required for secretion of proinflammatory chemokines under steady state conditions.

Conclusion: Soluble factors from fibroblasts are essential to create *in vivo*- like barrier properties in organotypic small intestine models constructed from either Caco2-HT29 cell-lines or primary duodenum epithelial cells. This emphasizes the importance of small intestine lamina propria when developing gut models for incorporation into multi-OoC.

Presentation: Poster

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Endotoxin contamination alters macrophage-cancer cell interaction and therapeutic efficacy in pre-clinical 3D *in vitro* models

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Endotoxins or lipopolysaccharides (LPS) are found in the outer membrane of gram-negative bacteria and have profound *in vivo* responses. Toll-like receptor 4 (TLR4) is the lipid A inflammatory signal transducer responsible for the TLR4 signal pathway induction [1]. They can trigger strong immune responses and therefore are unwanted contaminants in (bio)materials. Their activity must be as low as possible. Hence, FDA defined 2.15-20 EU/medical device or 0.06-0.5 EU/ml as limits depending on the type of application.

The rapid developments in biofabrication, in particular 3D bioprinting, but also the developments of organoid and organ-on-chip systems for drug testing, in the recent years have facilitated the need for novel biomaterials. The main focus in the development of biomaterials is on replicating the target tissue's composition, mechanical properties and providing cells with the optimal environment to proliferate, differentiate and communicate. A potential endotoxin contamination has been often overlooked.

In the last years, it has become evident that tumor-associated macrophages in the tumor microenvironment along with other immune cells such as T cells, play a crucial role in the progression, invasion and development of drug resistance in several cancer types [2].

In this study we demonstrate the effects of endotoxins in commercially-available gelatins on the macrophage (RAW264.7) – cancer (4T1) cell crosstalk in a 3D bioprinted co-culture model by measuring relevant macrophage and cancer cell biomarkers.

We observed that, while having the same mechanical and structural hydrogel properties, high levels of endotoxin can have significant influence on the metabolic activity of macrophages and cancer cells. Furthermore, this study shows that high endotoxin contamination causes a strong inflammatory reaction in macrophages and significantly inhibits the effects of a paracrine macrophage-cancer cell co-culture. It was also demonstrated that the differences in endotoxin levels can drastically alter the efficacy of novel macrophage modulating immunotherapeutic drug, 3-meth-

yladenine. Which might lead to misinterpretation of the potency and safety of novel immune therapeutic compounds.

References
[1] Diamond et al. (2015). *Immunotargets Ther 4*, 131-141.
[2] Binnewies, M. et.al. (2018). *Nat Med 24*, 541-550.

Presentation: Poster

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Ototoxic effects of cisplatin and gentamicin in human inner ear organoids and human adult vestibular organs

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Hearing loss and balance disorders can result from inner ear damage after treatment with antitumor drugs or antibiotics. Current animal and *in vitro* models do not completely represent the human inner ear; therefore, safety testing and identification of pathophysiology remain challenging. Recently, inner ear organoids (IEO) derived from human pluripotent stem cells (hiPSCs) have been described [1,2]. These organoids contain cochlear or vestibular hair cells [2,3], which are morphologically and physiologically similar to their counterparts in the normal inner ear.

Using IEOs and human adult vestibular organs, we aim to elucidate the pathophysiological mechanisms underlying the ototoxic effects of the antitumor drug cisplatin and the antibiotic gentamicin.

To this end, IEOs were generated from hiPSCs in three-dimensional culture and, next, 200 µm thick vibratome sections were obtained at day 75 to access the hair-cell-containing inner ear vesicles within the organoids. The ototoxic compounds were applied for 48 hours; cisplatin doses ranged from 0-2 mg/ml and gentamicin doses from 0-100 µM. Immunofluorescence was used to evaluate stereocilia bundle presence, apoptosis marker expression and neuronal integrity in the treated and untreated samples. These findings were compared with human vestibular organs obtained during translabyrinthine vestibular schwannoma surgery and subjected to identical treatments.

Preliminary results indicate increased apoptosis within the inner ear vesicle epithelium of the vibratomized IEOs and loss of neuronal integrity after gentamicin treatment. Ongoing work focusses on the time course of these changes and the ototoxic effects of both compounds on the human adult vestibular organs and the human IEOs.

References
[1] Van der Valk, W. H. et al. (2022). A single-cell level comparison of human inner ear organoids and the human cochlea and vestibular organs. *Preprint on bioRxiv*.
[2] Koehler, K. R. et al. (2017). Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. *Nat Biotechnol 35*, 583-589.
[3] Moore, S.T. et al. (2022). Generating high-fidelity cochlear organoids from human pluripotent stem cells. *Preprint in Cell Stem Cell*.

Presentation: Poster

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Establishing a breast cancer organoid panel to assess drug response in an underserved South African patient population

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Background: Cancer care for African women is mostly based on data gathered in the global west resulting in underrepresentation of African women. This bias has real consequences in adverse effects of treatments, problems with diagnoses, and treatment choices. A major problem to develop Africa-centric cancer care is the lack of appropriate preclinical models for investigation. In this study we establish a panel of breast cancer organoids from African breast cancer patients with a view to identify markers that can be used to assist in chemotherapy treatment decisions and to identify novel genetic alterations and physiological differences that may inform diagnostic and treatment developments.

Methods: Resected tissue obtained from mastectomies are used to establish organoids and gain genomic data while blood samples deliver germline genomic data. Organoids are exposed to the currently available chemotherapies within the public health system

and genomic and proteomic data is gathered to correlate to drug sensitivity. Furthermore, cell biology and biochemistry are performed on organoids of interest to further investigate potentially novel pathways or alterations leading to cancer progression.

Results and discussion: We have successfully implemented an isolation and processing protocol to establish breast cancer organoids from black African patients. These have been essayed for their sensitivity to the available chemotherapy drugs and patient follow up will be performed over the next decade to ascertain patient survival and cancer progression. Genomic and proteomic analysis is starting to reveal targets of interest while a unique cancer lacking cell-substrate adhesive capability is being investigated to identify the protein responsible.

Conclusion: With the establishment of the African breast cancer organoid panel we have created a resource for researchers to use when developing Africa-centric cancer care strategies and for understanding breast cancer in African patients. Our results will further assist in making improved treatment decisions avoiding unnecessary burdens on vulnerable patients.

Presentation: Poster

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Traumatic nerve injury models for drug development

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Day to day life can lead to traffic accidents, injuries at the workplace, incidents at home or during hobbies. Consequences can be devastating and include complete loss of motor function or chronic neuropathic pain due to nerve damage. Peripheral nerves are made of motor and sensory nerves, two very distinct types of neurons that are linked but each have their specific function.

Micro-Physiological Systems (MPS) offer the advantage to isolate neuron somas from their axons, thus reproducing the human anatomical architecture and enabling injury or treatment paradigms aligned with real life situations. To tease apart each cell type and allow their study separately, we adapted the culture of motorneurons and sensory neurons onto our MPS platform. To bridge the gap between *in vivo* models and first-in-human studies, as well as increase relevance, we developed our models using hiPSC-derived neurons.

As a mirror of current *in vivo* models, such as nerve crush injury or nerve ligation that aim to mimic human nerve trauma, we created a repeatable and standardized injury, by cutting, motor or sensory axons only using a short, targeted detergent application. The specific shape of NETRI's DuaLink Delta Ultra chips and our live staining protocol, combined with the high-throughput format of the NeoBento allow to easily monitor the neurite outgrowth dynamics during the whole period of drug application. We validated our models by comparing axonal regeneration following treatment with neurotrophic molecules or drugs inhibiting neurite outgrowth. To summarize, we offer pharmaceutical companies and researchers a new translational model of traumatic nerve injury to study efficacy and mode of action of novel therapeutic modalities.

Presentation: Poster

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Comparison of *in vitro* oral and topic absorption toxicity of BPA and BPS using 3D cell cultures and microfluidic systems

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Organ-on Chip is an effective solution to pursue new methodologies for drug discovery, toxicity tests and personalized disease treatments. It has been applied as an efficient and predictive solution to beat the high cost of classic toxicity tests using animal models, also reducing the number of animals in experimentation. The development of tools integrating different tissues, dynamic cell environments and cellular communication to the expression of high-fidelity organ function have allowing the setup of test assembly increasing predictability. Here we propose a junction of three different 3D tissue engineered cultures (skin, intestine, and liver) in a 3-organ-on chip microfluidic device to verify topic and oral Bisphenol A (BPA) and Bisphenol S (BPS) administration. After treatment we evaluate liver toxicity and endocrine disruption. For this, we developed models of human reconstituted skin, intestinal barrier, and liver spheroids, which were characterized in terms of histology, morphology and functionality. Our results show that our models are functional and simulate functions of the real organs. The Chip integration of all the tissues on the chip was well succeeded and improved viability of the 3D cultures. After treatment with Bisphenol A, we observed absorption of drugs, which caused liver injury and genetic modulation of endocrine disruption pathways, as expected. Interestingly, for BPS, which was reported as a substitute for BPA, decreasing toxicity and BPA damage, also induced toxicity and genetic modulation of this markers. In conclusion, here we present a new methodology to screen liver toxicity avoiding animal testes in two contexts, oral and topic administration of drugs. In addition, we found BPS also causes toxicity in liver and also can be a endocrine disruptor.

Presentation: Poster

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Biofabrication of long-lasting perfusable human vascular tissue on chip to investigate effect of fluid flow on vessel remodeling

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Integration of functionally matured vasculature is one of the major restrictions to size and complexity of engineered tissue constructs. Increasing demands for comprehensive on-chip micro(patho) physiological models is therefore, fundamentally intertwined with vascularization and proper perfusion of synthetic tissues. However, in-lab engineering of microvasculature, with physiologically relevant perfusion capabilities, requires thorough understanding of the physics underlying how network morphology correlates with the flow field. In physiological vessel development, onset of blood flow triggers a series of remodeling processes which render structural maturation of the primitive vascular plexus into a hierarchical network of vessels. Through vascular remodeling, the network architecture is normalized toward a more organ-specific structure which better meets metabolic needs of the tissue. Vessel remodeling has long been identified as a crucial contributing factor to functionality of vascular networks and tissue perfusion. However, how it is not yet well understood how differential network morphologies affect overall vessel transport properties. We have previously shown that vessel network density can negatively affect efficiency of blood distribution in murine retinal vasculature. Our coupled *in vivo* and *in silico* network and flow analyses showed that, branching complexity negatively affects blood perfusion efficiency and tissue oxygenation during neovascularization. These results, contradicting the conventional thinking that higher branching density correlates with higher perfusion, show the need for a quantitative evaluation of network transport properties considering vessel 3D branching pattern. Prompted by these results, we have developed an *in vitro* model of human vasculature-on-a-chip to further investigate the underlying physics of flow-induced adaptive vessel normalization. Our model, comprising human primary endothelial, mural, and stromal cells, exhibits long-term perusability up to several days with maintainability for over a month. Taking advantage of a non-invasive imaging method for real-time analysis of microvascular growth under constant perfusion, here, we present results of our quantitative analyses of vascular adaptive remodeling under flow. Our findings will contribute to the next generation of vascularization platforms for tissue engineering as well as 3D models for in-depth studying of vessel morphogenesis under flow.

Presentation: Poster

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3D printed microphysiological systems for 3D tumor culture, personalized anti-tumor drug screening, and tumor metastasis

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Recent legislation passed by the US House to end FDA animal drug testing, urgently demands non-animal based *in vitro* functional drug testing models. Moreover, less than 4% of cancer drugs get FDA approval, indicating the failure of current animal-based drug discovery models that do not mimic the human tumor microenvironment faithfully. Hence, the last decade witnessed numerous tumor-on-chip models based on spheroids, organoids, biopsy tissues, slices, and 3D bioprinting. However, previously reported models were fabricated by photolithography which has a high turnaround time. Hence, we report the additive manufacturing of three novel 3D tumor-on-chips and their applications in 3D tumor culture (versatile 3D tumor-on-chip (V3ToC)), precision medicine (personalized tumor-on-chip (PToC)) and predicting cancer metastasis (personalized metastasis on chip (PMoC)) with primary oral tumor cells and cell lines.

All three devices could be rapidly fabricated within 3-4 h in a two-step process, namely (1) stereolithography (SLA) of molds and (2) Casting of PDMS and assembly. 3D cell lines culture in the form of tumor spheroids, gel encapsulation, 3D cell patterning, and 3D bioprinted tissues in V3ToC showed excellent viability for 3 days. Further, the device will be validated with primary hepatocytes for drug toxicity and 3D printed primary lung tumor cells for drug efficacy.

Besides, patient-derived oral tumor spheroids remained highly viable for 5 days in the device (PToC), indicating excellent biocompatibility of SLA molds. Dissimilar drug screening results were observed across three oral tumor patient-derived spheroids tested with clinically relevant dosages of paclitaxel (1 μM), 5 Fu (20 μM), and cisplatin (5 μM) with 2 μl/min flow rate. We also found that the drug responses matched the histopathological reports of each patient (2 well-differentiated oral squamous carcinoma patients and 1 moderately differentiated oral squamous carcinoma patient).

We demonstrate that 3D printed molds can also be used to fabricate microposts-based 3 channel devices (PMoC) for hydrogel patterning and predicting oral tumor metastasis to the bone microenvironment.

Our findings indicate that 3D printed soft-lithography molds can be a promising alternative for the rapid and facile fabrication of PDMS based tumor on chips for precision medicine, 3D culture, and cancer metastasis.

Presentation: Poster

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Immune-competent gut microphysiological system for host-microbe-immune interactions

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Gut microbiome has emerged as a key factor in human diseases including infectious diseases. Lacking, however, is an *in vitro* platform that can recapture the interaction of human colonic and bacterial cells under physiologic microenvironment. Here, we established a GuMI physiomimetic platform that can maintain key features of this microenvironment including mucosal barrier, oxygen gradient, nutrient feeding, and flow. Co-culture of organoids-derived human colon epithelia and oxygen-sensitive commensal bacteria indicates that GuMI can maintain the growth of obligate anaerobes without compromising the barrier function for up to four days. RNA sequencing analysis revealed that GuMI recapitulates cell responses to hypoxia and several gut commensals predominant in human fecal microbiota. We further successfully incorporated innate immune cells into GuMI and were able to maintain long-term co-culture. Multiplex cytokine assays suggested that the presence of innate immune cells activates the systemic immune responses to commensals. In summary, GuMI physiomimetic platform faithfully recapitulate colon mucosal microenvironment and can be a useful tool to study interactions of host mucosa with microbiome and pathogens.

Presentation: Poster

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A skin-on-a-chip microfluidic platform to investigate neurovascular interplays in rosacea

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By combining tissue engineering with microfluidic technology, microdevices known as organs-on-chip have been developed in the past few years to provide more relevant *in vitro* models that mimic the microenvironment of living organs.

Essential for skin disease modelling and pharmaceutical research, various skin-on-a-chip models have been constructed. However, most models focus on co-cultures of fibroblasts and keratinocytes with endothelial cells to form a vascularized skin equivalent [1-3]. Our aim is to study specifically the role of innervation in a skin disease, rosacea.

To this end, we designed a two-layer PDMS device with a lower chamber containing a 3D skin equivalent and sensory neurons communicating through a physical barrier. Separated from the lower chamber by a porous membrane, the upper chamber consists of two separate microfluidic channels that deliver nutrients to the cells by diffusion of the medium across the membrane. These separate channels allow each cell culture to be supplied with specific media at different flow rates. It is important that the device allows not only microscopic observations, but also the collection of culture media and cell material for analysis, as well as the use of drugs to induce or prevent rosacea.

References

[1] Lee, S., Jin, P., Kim, Y. K. et al. (2017). Construction of 3D multicellular microfluidic chip for an in vitro skin model. *Biomed Microdevices 19*, 22.

[2] Varone, A., Nguyen, J. K., Leng, L. et al. (2021). A novel organ-chip system emulates three-dimensional architecture of the human epithelia and the mechanical forces acting on it. *Biomaterials 275*.

[3] Wufuer, M., Lee, G., Hur, W. et al. (2016). Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. *Sci Rep*.

Presentation: Poster

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Assessment of a smooth muscle cell maturation protocol for the analysis of contractile properties

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Cardiovascular diseases (CVDs) are the leading cause of death globally with 1.3 billion people of the world's population suffering from hypertension, causing a tremendous public health issue. Despite this global burden, preclinical drug development lacks a human relevant cell-based assay system employing human smooth muscle cells for the assessment of CVD related hypertension.

Here we assessed two aortic smooth muscle cell (HAoSMC) cultivation protocols and compared the maturation process of the cells on phenotypic level with immunostainings and on functional level with the FLEXcyte 96 technology. Primary HAoSMCs were cultured in T25 flasks for several days in recommended maintenance medium. After preculture, the cells were dissociated and plated on flexible membranes of the FLEXcyte 96 plates to ensure a human heart related physiological environment. Subsequently, the cells were cultured for 5 days in either maturation or maintenance medium before functional assessment of contractile properties. Here, compounds with positive inotropic effect (e.g., phenylephrine) were used for phenotypic characterization, the cells were fixed and stained with Alexa Fluor 488 Phalloidin directly within the plate.

The results demonstrate the effect of the maturation protocol on HAoSMCs with a stronger contraction (+30% compared to non-maturated control cells) and more pronounced actin structures. The assessment of an optimal preculture protocol for HAoSMCs ensures ideal conditions for further development. The use of HAoSMCs on the FLEXcyte 96 as standard cell model in near future will yield in a new standard cell-based assay system for the analysis of CVD related hypertension.

Presentation: Poster

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Establishing chemosensitive and drug resistant small cell lung cancer mini-tumor models using dynamic spheroid cultures

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Small cell lung cancer (SCLC) is aggressive, fast growing and easily metastasize with frequent relapse due to multi-drug resistance (MDR). MDR in SCLC is often associated with efflux transporter protein overexpression, including P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1). SCLC standard treatment has remained largely unchanged for the past three decades, despite lung cancer being one of the most common cancers diagnosed and SCLC having such a poor prognosis. In cancer drug development, however, the majority of new drug entities fail in clinical trials despite promising results during development. A major contributing factor to the low success rate is the lack of adequate and representative pre-clinical screening models. Three-dimensional (3D) cell cultures have increasingly been shown to better bridge the gap between traditional cell culture and *in vivo* models, and spheroids mimic non-vascularized tumors with cell-cell and cell-extracellular matrix interactions while their multi-layered structure result in nutrient gradients and hypoxia found in tumors. Spheroids are therefore useful in the development and screening of new drug entities but should be fully characterized and validated for such applications.

Dynamic chemosensitive and drug resistant (MRP1 hyperexpressive) SCLC mini-tumor spheroids were developed in a clinostat-based rotating bioreactor system. Spheroid growth and viability were characterized for at least 25 days, the optimal experimental window identified, and applicability for anticancer treatment screening was evaluated by treating the spheroids with the standard chemotherapeutic drugs irinotecan, paclitaxel, and cisplatin. Parameters measured included soluble protein content, planar surface area measurements, intracellular adenosine triphosphate and extracellular adenylate kinase levels, estimated glucose consumption, histological morphology, and efflux transporter gene expression. Both models established proved viable and stable, and the chemosensitive NCI-H69V SCLC spheroid model presented with decreased viability following treatment with irinotecan, while drug resistance was observed against irinotecan (MRP1 substrate) in the NCI-H69AR model. The NCI-H69AR mini-tumor model also demonstrated resistance to treatment with paclitaxel and cisplatin. These models were viable, stable, and functional and were shown to be reactive to treatments resembling tumors found in patients and can be used to screen new drug entities for potential efficacy against chemosensitive and drug resistant SCLC.

Presentation: Poster

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Generation of 3D human lymph node organoids to study immune responses in multi-organ-on-chip models

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Background: Lymph nodes and tonsils are secondary lymphoid organs that are fundamental in orchestrating the adaptive immune response. They have a highly specialized architecture that is regulated by non-hematopoietic Fibroblast Reticular Cells (FRCs), which support immune cell functioning. The global study of human antigen and tissue-specific immune responses lacks robust lymph node organoid and multi-Organ-on-Chip (multi-OoC) models due to their complexity and high immune cell diversity, where the inclusion of stromal cells is also absent.

Aim: Here, we aim to create static 3D human lymph node organoid models that can be implemented into microfluidic devices for future multi-OoC models, and where the microenvironmental control of the adaptive immune response is truthfully represented by the inclusion of FRCs.

Methods: Primary human FRCs and immune cells were isolated from lymph node or tonsil biopsies. FRCs were co-cultured with immune cells or the DC-like cell line MUTZ-3 DC in 3D collagen-fibrin hydrogels for up to 4 weeks. At the end of the culture period, immune cell profiles were characterized through flow cytometry and cytokine/chemokine analysis, as well as histology and 3D imaging to visualize cellular localisation.

Results: The presence of FRCs in the hydrogel resulted in the drastic survival of immune cells, namely B cells in the immune cell co-cultures. This was more evident in tonsil compared to lymph node cultures and coincided with higher levels of stromal-secreted CXCL12 and BAFF. FRCs improved the viability of MUTZ-3 DC cells and were also found to influence the development of MUTZ-3 DC under inflammatory stimuli to a lymph node-resident DC-like phenotype. Imaging revealed direct cell to cell contact between FRCs localised with immune cells.

Conclusion: This model highlights the importance of FRCs for immune cells in a lymph node model. Such a platform presents opportunity to further study antigen and tissue-specific adaptive immune responses, ultimately in a microfluidic multi-OoC setting to recapitulate microenvironmental effects on immune cell functioning.

Presentation: Poster

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Development of an *in vitro* 3D endothelialised-skin-on-chip model for toxicology testing and immune cell trafficking

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Background: Most of the developed *in vitro* human skin models are cultured under static conditions, therefore lacking the dynamic medium perfusion which would enable a more physiological nutrient transport, flow of immune cells, and a more accurate representation of human physiology.

Aim: To develop an incubator-free microfluidic device for the integration and control of complex three-dimensional biological models.

Methods: The microfluidic system combines an adaptor device compatible with standard commercially available transwell inserts and multi-well plates with a microenvironmental control console, which allows for the regulation of biological parameters (temperature, shear stress, N_2, O_2, and CO_2 levels). To generate a Skin-on-Chip model (SoC), reconstructed human skin (RhS) was cultured in the device for up to three days and exposed to internal body temperature and medium flow on the dermal side, while ambient temperature air-exposure was achieved on the epidermal side. In a toxicology study, endothelialised-RhS were topically exposed for 24 hours to either water or the sensitizer $NiSO_4$. RhS viability was confirmed by LDH and MTT assays, while morphology was assessed by immunohistochemistry on fixed tissue sections. To mimic immune cell trafficking in the blood, MUTZ-3 ($CD14^+/CD34^+$) progenitor cells were flowed underneath the exposed RhS and their viability and maturation were assessed by flow cytometry.

Results: In the SoC, we were able to maintain a sealed interface between the air and liquid compartments, did not experience RhS shrinkage, and were able to flow MUTZ-3 progenitors beneath the RhS. RhS displayed normal morphology and expressed epidermal differentiation and proliferation markers, in line with native human skin. We also demonstrated the ability of MUTZ-3

progenitors to flow and survive within all the compartments of the device for 24 hours. Moreover, the combination of an endothelialised-SoC and MUTZ-3 progenitors generated a physiologically relevant immune competent skin model which was used in a preliminary toxicology study to assess the effects of topical skin exposure of $NiSO_4$ on MUTZ-3 maturation and activation.

Conclusion: To our knowledge, we report for the first time an incubator-free microfluidic device allowing simultaneous user control over multiple physiological parameters for the combination of complex biological models and immune cell trafficking.

Presentation: Poster

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Modeling Nipah virus infection and treatment in a microfluidic lung-chip in maximum containment

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Nipah virus (NiV) is a zoonotic high-consequence pathogen that predominantly replicates within epithelial cells and macrophages of the respiratory tract and the central nervous system. There are no licensed therapeutics available for treatment of patients presenting with severe pneumonia and/or encephalitis resultant from NiV infection. Historically, modeling of NiV respiratory infection has been limited to animal experiments or conventional *in vitro* cell-culture-based assays that are unable to replicate the complex physiology of the lungs; this work is further complicated by the requirement of a maximum containment (BSL-4) laboratory. Therefore, we sought an alternative model that could reproduce the structural, functional, and mechanical properties of the lungs – including an air-liquid interface – and could be safely used in the BSL-4 laboratory. Toward this end, we adapted a commercial microfluidic lung-on-chip system, comprised of two microchannels, separated by a poly-dimethylsiloxane membrane barrier, previously employed to study Risk Group 2 and 3 pathogens [1-3], to examine NiV infection and test two small-molecule antivirals. After exposure of human donor-derived small-airway epithelial cells to NiV, effluent was collected from the top (epithelial) and bottom (endothelial) channels to determine NiV infectivity, cellular inflammatory responses, and endothelial barrier integrity. We confirmed the presence of infectious NiV in both channels, indicating that replication significantly increased the permeability of the barrier between the two channels. We then evaluated two small-molecule antivirals with known (remdesivir (at 100 nM)) and unknown (zotatifin (at 50 nM)) inhibitory effects on NiV replication and measured a significant reduction in NiV infectivity as well as a noticeable restoration of the barrier after treatment. To our knowledge, this is the first description of a microfluidic lung-on-chip that emulates the complex lung physiology within a BSL-4 environment and models the NiV-lung interaction. Our model may pave the way for similar applications of advanced microphysiological systems for the study of other Risk Group 4 viruses and additional evaluations of antiviral therapies.

References

[1] Hu et al. (2010). *Science 328*, 1662-1668. doi:10.1126/science.1188302

[2] Si et al. (2021). *Nat Biomed Eng 5*, 815-829. doi:10.1038/s41551-021-00718-9

[3] Zhang et al. (2021). *Adv Sci (Weinh) 8*, 2002928. doi:10.1002/advs.202002928

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Microphysiological systems: An emerging model for cancer research

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Introduction: Cancer is one of the fastest-growing causes of death worldwide, second only to cardiovascular-related diseases. This high level of burden reveals the importance of innovative approaches to research around its treatment. In recent years, many novel approaches have been deployed in cancer research. One such is the human Microphysiological Systems (hMPS), otherwise known as organ- and tissue-on-a-chip models. The system emerged to bypass the challenge around the two-dimensional cell culture approach. This study analyses the current evidence for this new technology, the challenges and limitations facing its use and future opportunities around it.

Methodology: Searched for relevant literature between December 16 and December 30, 2022, published in PubMed, Ovid MEDLINE, ScienceDirect, and Embase. All articles considering human Microphysiological Systems (hMPS) and cancer research were included. Articles not published in English were excluded due to translation limitations.

Result: This work focused on providing an overview of new findings around the human Microphysiological Systems (hMPS),

significant challenges and limitations to its use and future opportunities that the scientific community in cancer research can employ.

Conclusion: Several studies demonstrated that human Microphysiological Systems (hMPS) are more beneficial than the traditional *in vitro* modalities due to their ability to mimic the human microenvironment closely. However, it is not without its challenges and limitations. One of the critical highlighted challenges is the demand for high-level training needed to operate this new technology. Despite this, human Microphysiological Systems (hMPS) have been demonstrated to be the new mainstay in cancer research.

References

[1] Kim, H. N., Habbit, N. L., Su, C.-Y. et al. (2019). Microphysiological systems as enabling tools for modeling complexity in the tumor microenvironment and accelerating cancer drug development. *Adv Funct Mater 29*, 1807553. doi:10.1002/adfm.201807553

[2] Dsouza, V. L., Kuthethur, R., Kabekkodu, S. P. et al. (2022). Organ-on-chip platforms to study tumor evolution and chemosensitivity. *Biochin Biophys Acta Rev Cancer 1877*, 188717. doi:10.1016/j.bbcan.2022.188717

Presentation: Poster

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Assay development of novel high-throughput *in vitro* assay system using microvascular-on-a-chip for the evaluation of oligonucleotide-induced platelet aggregation potential

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Severe thrombocytopenia has been reported in oligonucleotide therapeutics (Sewing et al., 2017), however, no *in vitro* evaluation system that can evaluate the safety risk on a high-throughput manner has yet been established. To mitigate serious thrombocytopenia risk, it is important to discover oligonucleotides without the risk in many candidate oligonucleotides efficiently. In this study, we developed novel high-throughput microvascular-on-a-chip system for the evaluation of oligonucleotide-induced platelet aggregation risk potential. First, in order to evaluate the aggregation activity of platelets, we prepared a microvascular-on-a-chip using OrganoPlate® (Mimetas) and tested whether the activity could be observed in the microvascular with mouse whole blood. As a result, while little platelet adhesion to vascular endothelial cells was observed in the vehicle group, platelet aggregates were observed concentration-dependently in the collagen-treated group. In addition, collagen-induced platelet aggregates were suppressed by pretreatment of SYK pathway inhibitor which inhibited platelet activation. Then, ODN2395, which has been reported to have platelet aggregation activity via the GPVI pathway (Flierl et al., 2015), was evaluated in this assay system, resulting that platelet aggregate formation and platelet adhesion to vascular endothelial cells were observed concentration-dependently. Also, ODN2395 (PO), which has phosphodiester (PO) backbones and is considered to have no platelet aggregation activity, did not form platelet aggregates in this evaluation system. On the other hand, ODN 2395M, which was modified sequence and had binding activity to GPVI similar to ODN2395, did not form platelet aggregates. These results suggest that ODN2395 is involved in platelet aggregation by mechanisms other than GPVI. Together, the microvascular-on-a-chip system would be useful to mitigate oligonucleotide-induced platelet aggregation risk and to provide insight for elucidating the mechanism.

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Development and functional characterization of a microphysiological system for assessing *in vitro* drug toxicity and metabolism in the hepatobiliary environment

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The liver is a key organ for the metabolism of exogenous molecules, such as new drugs. Currently, the preclinical studies to evaluate the toxicity and safety of these new compounds rely upon *in vitro* and *in vivo* models according to the regulations [1]. However, these models cannot completely reproduce the complexity of the human liver's metabolic pathways, thus failing to fully predict the possible outcome in clinical studies [2]. In recent years, advanced *in vitro* approaches appeared on the scene as promising candidates to better simulate human liver physiology and, therefore, improved models for the human response. Microphysiological systems, namely liver-on-chips, are recognized as one the most encouraging *in vitro* approaches to reproduce the microarchitecture and functionality of the human liver [3]. In this study, a liver-on-chip has been developed allowing the culture of human hepatic, endothelial, and biliary cells. The device consists of several silicon layers that are interposed by porous membranes and where the flow is actuated thanks to a rocker device. Indeed, the proposed model was studied in static and dynamic conditions with the characterization of the main liver functionalities (e.g., albumin production). Based on the obtained results, a culture implemented with the three cell types and in the presence of the flow allows better liver functionality compared to the simple culture of hepatocytes and endothelial cells in static conditions. Therefore, the developed liver-on-chip could be considered a potential *in vitro* platform for toxicity and drug metabolism studies during the preclinical process.

References

[1] Avila, A. M. et al. (2020). An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs). *Regul Toxicol Pharmacol 114*, 104662. doi:10.1016/j.yrtph.2020.104662

[2] Messelmani, T. et al. (2022). Liver organ-on-chip models for toxicity studies and risk assessment. *Lab Chip 22*, 2423-2450. doi:10.1039/d2lc00307d

[3] Telles-Silva, K. A. et al. (2022). Applied hepatic bioengineering: Modeling the human liver using organoid and liver-on-a-chip technologies. *Front Bioeng Biotechnol 10*, 845360. doi:10.3389/fbioe.2022.845360

Presentation: Poster

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Modelling natural killer cell development in a microfluidic bone marrow model

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The human bone marrow (hBM) is a complex organ critical for self-renew and differentiation of hematopoietic progenitor cells (HPCs) to various lineages of blood cells including erythrocytes, leukocytes, and platelets. Perturbations of the hematopoietic system have been reported to cause numerous diseases. Yet, understanding the fundamental biology of the hBM in health and disease and during preclinical stages of drug development is challenging due to the complexity of studying or manipulating the BM in humans. Human cell-based microfluidic bone marrow models are promising research tools to explore multi-lineage differentiation of human stem and progenitor cells over prolonged time spans ranging from days to weeks recapitulating the physiologic process. Currently these systems focus mostly on tracing and quantifying erythroid and myeloid cell development and less on lymphoid differentiation.

Here, we describe for the first a time a microfluidic bone marrow model focusing primarily on natural killer (NK) cell development, cells that play a role in the innate immune response. Human hematopoietic stem and progenitor cells were cultured in a lymphoid cultivation medium in coculture with mesenchymal stromal cells on a zirconium oxide ceramic scaffold. The kinetics of differentiation into mature NK cell was traced by flow cytometry over a period of up to seven weeks. Alongside with the NK cells, myeloid cells developed in the system including granulocytes, monocytes, and dendritic cells. The differentiated NK cells could be activated after stimulation with phorbol myristate acetate (PMA) and ionomycin indicating the functionality of the cells.

In summary, the presented model enables investigation of human NK-cell development in the bone marrow and builds a basis to study related diseases and drug response effects in a physiologically relevant human microenvironment.

Presentation: Poster

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3D *in-vitro* breast models to understand the role of stiffness on breast cancer cell stemness and bone metastasis

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Increased breast tissue stiffness is correlated with breast cancer risk and invasive cancer progression. We previously observed that MDA-MB-231 breast cancer cells experiencing mechanical stresses due to increased stiffness of the extracellular matrix (ECM) exhibit changes in phenotype such as stemness [1]. However, the role of primary tumour stiffness in promoting bone metastasis, a major cause of mortality, is not yet understood. New 3D microphysical systems are designed to control elasticity of matrix and transmission of forces as *in vivo*. These can help in isolating the breast-bone axis and decipher the effect of mechanically conditioned breast cancer cells on bone tissue invasion and metastases. In this study, we have assembled two distinctive 3D scaffolds to model the breast-bone axis: an alginate-based hydrogel encapsulating human breast cells to model the tumour breast microenvironment; and polycaprolactone (PCL)-based 3D-printed scaffolds pre-conditioned with human osteoblast to model the normal bone tissue [2].

A small library of alginate-based hydrogels was characterized in compressive moduli (2-10 kPa) and protein content to mimic the properties of the breast tumour microenvironment and culture MDA-MB-231 cells. In parallel, different PCL-composite scaffolds (including bioceramics as particles) were 3D printed to mimic bone tissue stiffness and porosity. SaOs-2 cells activity was used to select the optimal scaffold, evaluating the deposition of bone-ECM and calcification. Selected bone-mimicking scaffolds were assembled with hydrogels breast scaffolds to create a new biohybrid 3D scaffold to study the breast-2-bone metastasis axis.

We observed that MDA-231 cells migration and 3D collagen invasion was higher when they were cultured in stiff hydrogels. Additionally, markers implicated in bone metastatic cascade, PTHrP and IL-6, were highly expressed in cancer cells that migrated from stiff hydrogels (breast) to biohybrid PCL scaffolds (bone) and not when cultured on tissue culture treated plates. Unlike *in-vivo* models, the presented *in-vitro* model could isolate and evaluate far reaching impact of tumour stiffness (a factor in early tumour progression) on later stages of metastatic cascade. These models support the paradigm shift of using MPS in enabling insights on metastatic pathways and towards new therapeutic targets [2].

References

[1] doi:10.1016/j.actbio.2022.08.074
[2] doi:10.1002/adhm.202201898

Presentation: Poster

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A 3D colon on chip to study the peristalsis influence on the cellular ecosystem in physiopathological conditions

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The digestive system enables nutrients absorption and represents a barrier against environment. The intestine is shaped with villi and crypts and only with crypts in the colon, stem cells being at the crypts bottom. It is also a dynamic organ subjected to peristalsis. Here, we aim at developing a new 3D gut-on-chip to unravel how peristalsis affects the intestinal ecosystem.

For this, we worked with a collagenI/PEGDA interpenetrating network (IPN) based hydrogel that can be stretched, 3D-molded and is compatible with epithelial and stromal primary cells co-culture.

We first optimized the IPN synthesis (Munoz-Pinto et al, 2015) and demonstrated the viability of organoïds and mouse intestinal fibroblasts (MIF). After 14days, high cell viability rates were recorded in IPN (70 ± 5.2% and 82 ± 1.6% for MIF and organoïds). The mechanical properties were deeply examined. The IPN retains its mechanical properties and shape even after 8000 stretching cycles (8% deformation, 0.1Hz). The stiffness increases with the IPN compared to collagen (237 ± 23.6kPa vs 85.7 ± 48kPa). For the cycling, both hydrogels lose stiffness but tend to stabilize, the IPN remaining stiffer than the collagen (≅40kPa vs ≅≅10kPa).

We next studied the effect of stretching on the colon-like ecosystem. We evaluated the proliferation (Ki67), the differentiation (RTqPCR) and the polarization of epithelial cells under stretching vs static conditions (1 day & 6 days). The stromal compartment was also examined. The stretching allows an increase of the proliferation and an enrichment of the stem cell compartment. However, no differences in the expression of differentiated epithelial cells were recorded. We quantified the cellular elongation and concluded that the stretching improves the polarization. Concerning the stromal compartment, the fibronectin production increased with stretching. These results highlight the importance of the peristalsis in the maintenance of a functional epithelium with a highly active pool of stem cells and a stroma that secretes new ECM.

This gut-on-chip is a critical step in the development of biomimetic models, combining for the first time the main gut characteristics. We are now expanding to pathological conditions (tumoroids/CAF) to study the influence of the microenvironment and mechanical constraints on tumor invasion.

Presentation: Poster

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The importance of traceability in dimensional metrology in microfluidic systems

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Microfluidics is the science that studies the behavior of fluids through pathways with micrometric dimensions and their manufacturing technology. Microfluidic devices are small, simple, portable and can be used to foster emerging applications such as microphysiological systems, organs-on-chip (OoC), drug development, lab-on-a-chip (LOC), etc. But much is still necessary to successfully connect the microfluidic devices to the world.

The work consisted in verifying connectivity compatibility in three microfluidic systems. The study, done in 3 microfluidic devices, concentrates on the measurement of dimensions of connection ports, connectors, and tubes and of volumetric flow rate. The dimensions were determined by interferometry traced to national length standards at IPQ and by profile projection. And the volumetric flow rate imposed on these systems was studied using the gravimetric method and the front tracking method, traced to national volumetric flow rate at IPQ. The later was also used to assess leakages in the microfluidic path.

The comparison between manufacturers' specifications and dimensions measured allowed inferring which method had the highest accuracy and lowest uncertainty for the characterization of this type of systems. This work also highlighted the need for harmonization in manufacturing of components and for metrological specification values traced to practical realization of SI units.

The outputs will be included in the normative framework to improve the manufacturing process and the quality control of microfluidic systems. This is expected to contribute to the development of standardized connection ports, connectors, and tubes towards the improvement of mimetization and basic understanding of microphysiological processes.

Presentation: Poster

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Modeling the role of the fallopian tube in the prevention and spread of high-grade serous cancer using a multi-organ platform

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Ovarian cancer is the 5th leading cause of cancer related death in women. If detected early, the chance of survival is over 90%, but most women are diagnosed in the late stages of disease. It is now recognized that a large percentage of high grade serous ovarian cancers arise in the fallopian tube and colonize the ovary as the primary metastatic step. One of the risk factors associated with ovarian cancer is the lifetime number of ovulations. Few models are currently available that allow the process of ovulation and the development of ovarian cancer to be studied. To address this gap, our team developed a microphysiological platform called PREDICT-multi-organ-system (MOS). PREDICT-MOS contains 30 wells for culturing organs and 60 pumps with precisely controlled flow rates. Human fallopian tube tissue can be grown in an air-liquid interface, while murine ovaries can also be grown in the platform and stimulated to ovulate and produce hormone and secretion profiles. Importantly, these secreted factors are contained in the device within the media and can therefore precisely model local concentration gradients and temporal expression. First, we uncovered that the ovary secretes versican during the cycle to enhance ovarian migration, adhesion, and expansion within the ovarian microenvironment. We revealed a role for high testosterone found in polycystic ovarian patients to stimulate stem cell expansion and invasion of fallopian tube cells. Next, we profiled the expression of secreted extracellular vesicles that are produced by the fallopian tube on the platform. Extracellular vesicles may provide a new form of liquid biopsy, but understanding the cargo expressed from normal cells is critical for understanding the importance of cancer EV's during progression. Finally, we have engineered the platform allow for real-time invasion using an insert technology, a modified pump that allows for interaction between the organs, and an agarose mold to enable the on-platform culture of 3D spheroids. Overall, the integration of MPS technology with key human tissues and engineered organs allows for the in-depth analysis of the role of ovulation in the onset and spread of high-grade serous cancer from the fallopian tube.

Presentation: Oral

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The development of a micro-physiological system of the human corneal epithelium under dry eye-like conditions

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Dry eye (DE) is a condition characterized by a lack of sufficient lubrication and moisture on the surface of the eye. This can lead to symptoms such as redness, itching, burning, and even visual disturbances. Modeling such a disease *in vitro* is difficult due to the lack of biological and biomechanical conditions. Previously, we have developed corneal epitheliums-on-a-chip (CEpOC) under eye-like blinking stimulus that could also allow the spatiotemporal analysis of metabolites secretion/transportation and their correlation with significantly expressed transporters in the barrier [1,2]. Herein, we used CEpOC for modeling DE by applying cycles of air-liquid interface. We then conducted RNA sequencing (RNA-Seq) of human corneal epithelial cells to investigate cell phenotypes under applied DE conditions as well as in combination with the nonsteroidal anti-inflammatory drug Diclofenac (DCF) that has a debatable safety result in the clinical practices. Pathway analysis of the differentially expressed genes (DEGs) revealed significant changes in the gene expression of key DE-related genes such as the overexpression of collagen (COL1A2 and COL3A1) and inflammatory cytokines (IL6 andIL7R). In contrast, there was a downregulation in the gene expression of adherence junctions (CDH1/2/3/16), lubricant mucins (MUC2/4/6), and cytokeratin filaments (KRT5/7/19). Although DCF did not have any significant effect on cell viability in both control and DE condition, however, on the transcript expression level, DCF treatment led to an over-expression of the pro-inflammatory cytokine IL-6, and a down-regulation of growth factor TGF-β1, occludin tight junction (OCLN) as well as the antioxidant glutathione synthetase (GSS). This suggests that DCF may have an impact on the integrity of the corneal barrier under the DE condition and hence it can cause a safety concern. In summary, by the application of air-liquid cycles in the CEpOC we could recapitulate a DE-like phenotypic changes in cells where we could test the *in-situ* toxicity and efficacy of DCF as a clinically relevant drug model. Thus, the CEpOC platform has the potential as a biomimetic DE model.

References

[1] Abdalkader, R. and Kamei, K.-I. (2020). *Lab Chip 20*, 1410-1417.
[2] Abdalkader, R., Chaleckis, R. and Wheelock, C. E. (2021). *Exp Eye Res 209*, 108646.

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In vitro metabolism of the ^{14}C-labelled fungicide tebuconazole by rat liver organ-model

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The usage of an organ-model gives the possibility to simulate the complexity of living organisms on a new level. It is conceivable to use organ-models to simulate the metabolism of xenobiotic compounds such as, pesticides or cosmetics. Currently, the metabolic degradation of pesticides is investigated by means of *in vivo* studies following the OECD guidance 417, which is needed for the registration of a pesticidal product [1]. A first evaluation, to mimic the metabolic competence of a liver-on-a-chip model, was conducted with the ^{14}C-labeled fungicide tebuconazole by means of rat liver spheroids.

Primary and pooled female Han Wistar rat hepatocytes and respective stellate cells were pooled to create 3D liver spheroids. Each spheroid contains 5,000 cells in total, with 4,500 hepatocytes and 500 stellate cells [2]. The organ-on-a-chip technology by TissUse (TissUse GmbH, Berlin, Germany) was used to incubate 30 spheroids for 7 days with 10 µM ^{14}C-tebuconazole under a 50 Hz flow [3]. A static incubation in a 24-well plate under comparable condition was done simultaneously. The usage of a ^{14}C-labeled chemical enabled a complete mass balance and an analysis of all supernatants and cell extracts by mass spectrometry coupled with microplate scintillation counting.

All samples confirmed known phase I (e.g., hydroxylation) and phase II metabolites (e.g., glucuronic acid conjugates) of ^{14}C-tebuconazole and all of them were detected during the entire incubation period. The cell extract contains quantitatively more metabolites than the supernatant. A comparison between the static and dynamic system reveals no qualitative and semi-quantitative differences of the metabolic profile of ^{14}C-tebuconazole. Lactate dehydrogenase and albumin levels confirmed the viability and the functionality of the hepatic model over 7 days.

Comparing these results with *in vivo* data shows that rat liver spheroids are suitable to investigate the metabolism of ^{14}C-tebuconazole *in vitro*. Moreover, the excretion of the metabolites into the nutrition medium is an important aspect for future organ interactions in multi-organ chips.

References

[1] OECD (2010). Test No. 417: Toxicokinetics. OECD Publishing, Paris. doi:10.1787/9789264070882-en
[2] Kyffin, J. A. et al. (2019). *Toxicol In Vitro 55*, 160-172.
[3] Bauer, S. et al. (2017). *Sci Rep 23*, 8.

Presentation: Poster

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Thyroid-on-a-chip: An *in vitro* organoid device to test thyroid disruption

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The thyroid gland can be severely affected by ubiquitous environmental pollutants, commonly known as endocrine disrupting chemicals (EDCs) [1]. Currently, the safety of potentially new EDCs is poorly estimated, in part, due to the use of 2D cell toxicological assays, which often lack physiological relevance. New and more advanced cell culture models are thus needed to better reproduce the thyroid architecture and the human EDC responses *in vitro*. Here, we demonstrate the development of a thyroid organoid-on-a-chip (OoC) device using polymeric, membranous carriers [2]. Thyroid follicles were differentiated from mouse embryonic stem cells (ESC) and incorporated in a continuous flow microfluidic device for a 4-day experiment at a flow rate of 12 µL/min. A reversible seal provided a leak-tight sealing while enabling quick and easy loading/unloading of thyroid follicles [3]. Oxygen levels in the medium were monitored throughout culture by integrating optic sensors on chip. Thyroid follicles in the OoC device maintained expression of key thyroid genes and exhibited a typical follicular structure with T4 synthesis at the luminal space. Short-term exposure of the OoC model to benzo[k]fluoranthene revealed alterations in gene and protein expression. In particular, transcriptomics showed changes related to activation of the xenobiotic aryl hydrocarbon receptor (AhR) pathway, alterations in lipid metabolism and on nuclear hormone receptors. Altogether, the data suggest that the novel OoC system is a physiologically relevant thyroid model, which represents a valuable tool for testing potential EDCs.

References

[1] Gore, A. C., Chappell, V. A., Fenton, S. E. et al. (2015). *Endocr Rev 36*, 593.

[2] Giselbrecht, S., Gietzelt, T., Gottwald, E. et al. (2006). *Biomed Microdevices 8*, 191.

[3] Carvalho, D. J. T., Moroni, L. and Giselbrecht, S. (2023). *Nat Rev Mater*. doi:10.1038/s41578-022-00523-z

Presentation: Oral

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Engineering a biomimetic glomerular filtration barrier chip for diabetic nephropathy modeling

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Diabetic nephropathy (DN) is a chronic microvascular complication of diabetes mellitus and is the leading cause of end-stage kidney diseases. One of the features of DN is proteinuria, protein leakage from the capillaries into urine caused by severe damage to the glomerular filtration barrier (GFB). Endothelial cells, glomerular basement membrane (GBM) and podocytes are the components of the GFB, and therefore, targets for the study of DN. While traditional DN models have offered valuable insights on GFB biology, they are oversimplistic, the interactions cell-microenvironment are obliterated and do not allow transport studies [1]. Besides tuning the ECM and stiffness of the substrate [2], recent evidence suggests that podocytes are responsive to topographical cues, such as microscale curvatures [3].

In this study, we developed and characterized a 3D microphysiological model of the GFB by culturing conditionally immortalized podocytes (ciPODs) on hollow fiber membranes (HFM, 500 µm outer diameter) mounted on a perfusable chamber. The HFM provides topographical guidance to the ciPODs whilst allowing for perfusion studies in which apical and basal compartment are accessible. Guided by the surface curvature, the podocytes tightly surround the engineered barrier, similar to the podocyte-capillary interaction *in vivo*. In particular, the cells show enhanced cellular processes, expressed known mature markers (nephrin, podocin), and increased GBM deposition (collagen-IV, laminin-5) when compared to the flat counterparts. Our model shows the size selectivity of the *in vivo* GFB, as proven by proteinuria assays based on library of different molecular weigths FITC-labeled dextrans. Healthy markers and sieving capacity appear disrupted upon DN conditions, induced by high-glucose exposure.

This novel model sheds new light on the the interactions and organization of podocytes to recognize or treat DN in early stages. The GFB-chip can be extended for elaborating safety, efficacy and

nephrotoxicity studies, as well as to mimic other glomerular diseases and screen for pharmaceutical compounds aimed at restoring GFB.

References

[1] Valverde, M. (2022). *Nat Rev Nephrol*.
[2] Yaoita, E. (2018). *Kidney Int 93*, 519-524.
[3] Korolj, A. (2018). *Lab Chip 18*, 3112-3128.

Presentation: Oral

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Culture of human lymph node fibroblastic reticular cells in different 3D biomaterials to mimic the lymph node T cell zone

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Lymph nodes are complex tissue-draining lymphoid organs that are essential for the initiation and maintenance of efficient adaptive immune responses. The non-hematopoietic lymph node stromal cells arrange the dense architecture of the lymph node and provide unique structural microenvironments that are specific for either B or T cells. In the T cell zone, fibroblastic reticular cells (FRCs) produce and ensheath various extracellular matrix (ECM) components forming a large FRC reticular network. This FRC network facilitates the survival, migration and encounter of immune cells within the lymph node, a process which is orchestrated by specific cytokines and chemokines. In our current studies we have used collagen-fibrin hydrogels to construct the T cell zone in a 3D plane. However, FRC network formation and immune cell migration is limited in these cultures. Therefore, studies into new 3D biomaterials for culture of the FRC reticular network are required. Here, we will compare different 3D biomaterials including functionalized peptide hydrogels, fibrous networks, porous network scaffolds and collagen beads, for the 3D culture of human FRCs. We will study FRC viability and specific surface marker expression including Podoplanin, CD90, CD34, HLA-DR and BST1. Furthermore, network formation and ECM production by FRCs will be investigated. Finally, cytokine/chemokine levels in the different 3D FRC cultures will be measured. Investigating different 3D biomaterials for the construction of the T cell zone will help the development of a human immunocompetent 3D lymph node which can ultimately be integrated in an organ-on-chip platform.

Presentation: Poster

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Engineering metabolically active reconstructed human skin for organ-on-chip

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Background: Considering its major barrier function, it is expected that the skin would be a metabolically very active organ. However, current reconstructed human skin (RhS) models do not adequately reproduce the metabolic potential of native human skin.

Aim: To determine whether the incorporation of an adipocyte containing hypodermis into RhS will improve its metabolic potential and to determine which major metabolic pathways are involved.

Methods: Primary human keratinocytes, fibroblasts and differentiated adipose-derived stromal cells (ASCs) were co-cultured to create an adipose-RhS (reconstructed epidermis on fibroblast populated hydrogel integrated above adipose layer).

Results: Lipid droplet formation, gene expression of key adipogenic markers and adipokine secretion confirmed successful differentiation of ASCs to adipocytes. Epidermal integrity was maintained. Addition of the adipose layer resulted in down-regulation of 9 and up-regulation of 286 genes in the dermal-adipose compartment compared to RhS with only the dermal compartment. Out of the up-regulated genes, 5 were identified as phase I, and 2 as phase II metabolic enzymes. Gene ontology analysis revealed that these were mostly involved in vitamin A and vitamin D metabolic pathways. The cytokine secretion profile changed drastically, showing reduced concentrations of pro-inflammatory cytokines in adipose-RhS compared to RhS.

Conclusion: Adipose-RhS has a less inflamed phenotype indicating the contribution of adipocytes to tissue homeostasis. Up-regulated phase I and phase II enzymes show higher metabolic activity of adipose-RhS compared to RhS. Therefore, adipose-RhS mimics native skin more than traditional RhS and hence is a better model for investigating human skin in health and disease in organ-on-chip.

Presentation: Poster

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Novel fully primary human airway epithelium-alveolar macrophages *in vitro* co-cultures models to study host pathogen interactions

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Being the first line of defense of the organism against airborne pathogens like bacteria and viruses, the respiratory epithelium acts as a physical barrier as well as an efficiency mucociliary escalator. Furthermore, the airway epithelium is also a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections.

Many animal models have been used to study lung infections, but the relevance and predictability of animal models are still questionable. Here we established a new co-culture model using well characterized, standardized human airway epithelium such as MucilAir™, SmallAir™ and human lung macrophages (CD45+, HLA-DR+, CD206+, CD11b+ and CD14-) for studying bacterial and viral infections. The alveolar macrophages were not only able to adhere to the epithelial cells, but also functional: The macrophages were capable of phagocytosis, evaluated using pHrodo™ Red (S cerevisiae Bio-particles Conjugate). Moreover, the co-culture models respond to pro-inflammatory stimuli such as LPS, TNF-a and Poly(I:C) with an increased IL-8 secretion.

Upon bacterial infection with methicillin-susceptible *Staphylococcus aureus* strain (MSSA), compared to MucilAir™ monocultures, MucilAir™-macrophages showed stronger immune responses: (i) a reduction of bacterial growth (up to 1.5Log10 CFU) and (ii) decreased upregulation of IL-8 and b-defensin-2 secretions. Interestingly, greater difference was observed for *Streptococcus pneumonia* (Sp19F): The presence of macrophages led to a decrease of 3.5Log10 CFU after 24 hours of culture (N = 12) versus MucilAir™ alone.

These novel *in vitro* models might find applications in understanding the role of immune-epithelial cell interactions in infection diseases.

Presentation: Poster

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Development of fully primary human 3D alveolar model (AlveolAir™)

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In order to develop novel drugs and assess lower respiratory toxicity of xenobiotics, robust and relevant *in-vitro* alveolar models would be very helpful. We herein describe the characterization and functionality of a full primary human epithelial-endothelial 3D alveolar model, AlveolAir™.

To characterize AlveolAir™, long-term parameters were measured: Biomarkers for ATIs, ATIIs and tight junctions (CAV-1, HTII-280 and ZO-1 respectively, Immunofluorescence); Morphology and lamellar bodies' presence (Histology & TEM); Existence of a maintained alveolar epithelial barrier (TEER) and SPC secretion (ELISA). Using these techniques, the culture evolution was monitored for several weeks.

Functionality of AlveolAir™ was evaluated by exposure to pro-inflammatory compounds (LPS, TNF-α, poly(I:C) and cytomix). TEER and cytotoxicity (LDH) were monitored daily, along with morphological observations and cytokines quantification. It revealed that AlveolAir™ can display an inflammatory response by secreting IL-6, IL-8 and RANTES significantly when treated with poly(I:C) or cytomix.

As proof-of-concept, effect of a flagellin-based formulation (inhaled drug candidate) was assessed on AlveolAir™. The tissues were exposed to 0.0003, 0.003; 0.03; 0.3 or 3 µg/cm^2 of flagellin apically 2 h/day, for 5 days. TEER, LDH, pro-inflammatory cytokines (IL-6 & IL-8) and a panel of genes were evaluated. The flagellin-based formulation had no effect on TEER and cytotoxicity for all tested conditions. However, flagellin did induce a dose-dependent increase of IL-8 starting at 0.03 mg/cm^2 and upregulate the expression of genes coding for CCL4, TNF, IL-1b, CSF3, or CCL20, with a plateau obtained at 0.03 mg/cm^2. Altogether, flagellin was well tolerated by alveolar epithelia. Apical exposure induced biomarkers upregulation, demonstrating flagellin's immunomodulatory potential on alveoli.

Finally, co-culture model between AlveolAir™ and primary alveolar macrophages has been developed. Upon bacterial infection with Streptococcus pneumonia (Sp19F) compared to AlveolAir™, AlveolAir™-macrophages showed stronger immune response with reduction up to 3.5Log10 CFU after 24 hours of culture.

This novel *in vitro* model, AlveolAir™, represents a relevant and reliable tool for inhalation toxicity assessment of drugs. It is also highly useful for understanding the cellular and molecular mechanisms of respiratory diseases such as COPD, viral and bacterial infections.

Presentation: Poster

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Remote magnetic alignment of spheroids in 3D matrix for muscle-on-chip

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Organizing cells anisotropically is essential to recapitulate the skeletal muscle tissue's 3D microenvironment. Most cells alignment methods rely on contact guidance cues [1], but alignment in 3D [2], especially in a gel, remains challenging.

Here we propose two innovative magnetic-based approaches for muscle tissue engineering [3]. The first approach generates magnetic spheroids as tissue building blocks, while the second one offers a new way to align magnetic cells or spheroids along a strong uniform magnetic field. Combined, the two-step process enables the on-chip creation of muscle fibers oriented in the magnetic field direction in collagen-based matrix. Cells were first labeled with iron nanoparticles, and optimization led to 21 pg of iron internalized per cell, with no impact on cell metabolic activity or capacity to differentiate. Microfabricated magnets could then attract the magnetically labelled cells and generate in 3 hours several thousand spheroids of controlled size (10-100 µm range). These spheroids could then be aligned on-chip in a 3D thermoresponsive collagen gel between two strong magnets. Once the gel polymerized, the chains of spheroids were trapped in this configuration. The chains were on average a few hundreds of microns long and could go up to 1 mm under optimal cell density and magnetic labeling. Such structures made with aligned spheroids remarkably maintained their anisotropy overtime. Besides, in a matter of days, the spheroid chains fused into fiber-like structures. These fibers could also be 20% stretched or co-cultivated in the 3D collagen gel with randomly dispersed fibroblasts, and several myofibers formed in the direction of the alignment.

Overall, this work demonstrates that combining magnetically assisted 3D strategies with organ-on-chip technology is beneficial for the fabrication of muscle tissue engineered constructs. As many tissues in the human body are anisotropic, these technologies to produce and align magnetic spheroids could open new perspectives for 3D tissue engineering.

References

[1] Jain, A. et al. (2021). *Nanomedicine 32*, 102341.
[2] Takeda, N. et al. (2016). *J Artif Organs 19*, 141.
[3] Demri et al. (2022). *Adv Funct Mater*, 2204850.

Presentation: Oral

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Assaying axonal damage and repair using microfluidics with fluid walls

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Spinal-cord injuries are a common cause of mobility impairment; damaged axons can no longer transmit electrical stimuli to peripheral muscles. However, there remains no pharmaceutical treatment promoting axon regrowth and electrical reconnection. A major reason for this is the scarcity of *in vitro* assays that combine facile ways of growing axons, introducing damage into them, and monitoring any regrowth. Whilst microfluidics has obvious potential, use of conventional devices made from plastics like polydimethylsiloxane (PDMS) bring challenges associated with performing such assays on axons inaccessibly buried behind solid walls.

Recently, an open form of microfluidics has been developed that enables fabrication of almost any 2D microcircuit in minutes on standard Petri dishes; the aqueous phase is confined by fluid (not solid) walls. These fluid walls are interfaces between the immiscible liquids: cell-growth medium and the fluorocarbon, FC40. These liquid walls prove to be remarkably robust, as dishes containing them can be carried about labs like any other dish filled with medium. They are also permeable to O_2 plus CO_2, and transparent; consequently, cells are grown in standard incubators and monitored microscopically as usual.

We now exploit the properties of such fluid walls to grow axons in dumbbell-shaped circuits in which one conduit (width ~200 µm, height ~25 µm, length 1 mm) connects two drops with square footprints (3 x 3 mm). Pressures in this circuit are governed by the Young-Laplace equation and can be tuned simply by changing drop volume. Knowledge of local pressures allows seeding of induced pluripotential cells (iPSCs) in one drop and brain-derived neurotrophic factor into the other without any spill-over into the conduit. Axons then grow into the conduit towards the factor.

Fluid walls are now removed without harming cells, axons cut using a submerged non-contact hydro-jet, and new fluid walls built. Regrowth of cut axons in the presence of test chemo-attractants is then monitored microscopically without optical distortion (as the refractive index of FC40 is close to that of water). This approach provides a simple and accessible way for biologists to assay axonal growth and recovery after damage.

Presentation: Poster

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Fabrication of 3D microstructures within a perfusable vasculature-on-a-chip system using two-photon polymerization

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Tissue engineering is an interdisciplinary research area with great potential for modeling human physiology and disease. One of the main challenges in this field is vascularization of *in vitro* grown tissues and organs. Although much research has been done in the area of vascular tissue engineering, it remains challenging to generate a fine and perfusable vasculature with appropriate mechanical properties and biocompatibility. Fine capillary structures can be formed *in vitro* by combining angiogenesis with 2-photon printing. Perfusion of an engineered vascular network is crucial to provide natural cell culture conditions that maintain optimal cell function. The lack of a functional and perfusable vascular system leads to an insufficient supply of nutrients and oxygen to cells, which restricts the dimensions and lifespan of an engineered tissue.

This work demonstrates a vasculature-on-a-chip device, which provides a vascular network for growing vascularized tissues. We outline the chip fabrication process, which combines stereolithography with two-photon printing. Stereolithography is used for printing main chip part, whereas a 2-photon technology aims to finalize the chip by printing 3D microstructures inside the channels and by providing reliable bonding in the areas that are hard to reach with a standard gluing technique.

In conclusion, we present a chip with 3D microstructures printed and imaged with a 2-photon technology, as well as material composition, biofunctionalization and testing with endothelial cells.

The creation of perfusable vascular structures covered with a functional endothelial cell layer would make a significant contribution towards the possibility of growing sizeable organ-mimicking structures with prolonged cellular survival.

Presentation: Poster

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Modeling inflammatory bowel disease in human intestinal organoids using a high-throughput workflow

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Inflammatory bowel diseases (IBD) are characterized by chronic inflammations of the gastrointestinal tract during which the intestinal mucosal barrier gets damaged. Until today, mice models have been used to unravel the complex interactions involved in IBD, yet they often fail to predict human responses. In recent years, organoids have emerged as a game-changing tool for disease modeling and drug screening. These organoids are three-dimensional, miniaturized, and simplified versions of an organ that mimic some of the key features of the native tissue *in vitro*. Traditional organoid culture methods embed these structures in solidified extracellular matrix (ECM), thus introducing an intrinsic lack of reproducibility and creating highly heterogeneous organoid populations. To overcome these challenges, we used Gri3D®, an innovative hydrogel-based ultra-dense U-bottom-shaped microcavity array platform. Gri3D enables the generation of a single organoid in each microcavity in suspension-like conditions, without a solid ECM, allowing organoid cultures standardization. Combined with a high-content imaging ImageXpress® Micro Confocal system, organoids were live-monitored over time to track key IBD-related phenotypes at a single-organoid level. We report the induction of intestinal inflammation on healthy human rectal organoids using pro-inflammatory cytokines (TNF-α and Il-1β). Upon treatment, the epithelial barrier was disrupted and further assessed by immunostaining of tight junctions. Interestingly, treated organoids show a slower growth rate and decreased budding capacity. We demonstrate the use of Gri3D as a robust and high-throughput *in vitro* platform for human GI organoid-based IBD modeling.

Presentation: Poster

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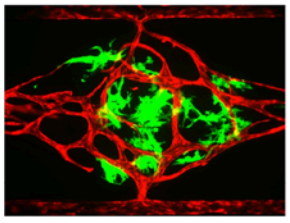
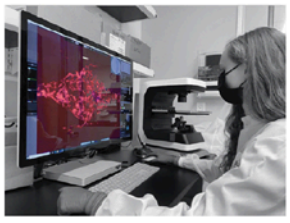


Oncology Studies



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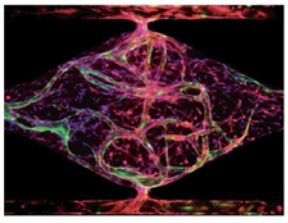
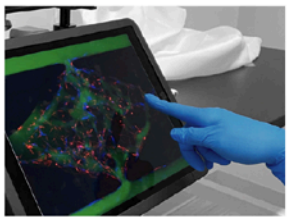


Blood-Brain Barrier Studies



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A novel approach for label-free drug efficacy analyses of pancreatic cancer organoids using high-content imaging

Marine Meyer[1], Maria Clapés[2], William Verstaeten[2], Aline Roch[1], Oksana Sirenko[3], Angeline Lim[3] and Nathalie Brandenberg[1,2]

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Cancer remains one of the leading causes of death in the 21st century. Despite the latest advances in oncology, the majority of cancer patients lack tailored therapeutic approaches with lasting benefits.

Measuring the impact of anticancer compounds and their combinations is only possible on *ex vivo* assays. However, fresh primary cell cultures remain challenging to establish for testing *ex vivo* in a clinically relevant manner both in terms of time and biological relevance.

To this end, patient-derived organoids (PDOs) have been proposed as viable and efficient alternatives for *ex vivo* testing. PDOs show long-term expansion potential while retaining tumor histopathology as well as cancer gene mutations. However, the translation of organoids in screening applications has so far been hampered by the lack of homogeneity and difficult handling and automatability. Moreover, they are randomly distributed across the culture which complicates subsequent readouts and image analyses.

To overcome these challenges, we set up a screening workflow on PDOs using Gri3D®, a ready-to-use platform for high-throughput and reproducible organoid culture. Based on a standard 96-microtiter plate, each well contains a microwell array patterned in a cell-repellent hydrogel. On Gri3D, organoids are robustly generated in microwells and located in the same imaging plane. This greatly facilitates quantitative analyses in high-content image-based screens. Furthermore, the pipetting port enables automation of cell seeding, media exchange, and compound incubation with liquid handlers, increasing assay reproducibility.

In the presented work, we exposed human pancreatic cancer PDOs to a panel of anti-cancer compounds at different doses and followed their response with brightfield images. Using an AI-based approach, we efficiently detected every single organoid and extracted phenotypic features which correlate with cytotoxicity. We further validated the approach by comparing the obtained results to a traditional multi-plane fluorescence-based Live/Dead assay.

The data demonstrate on human pancreatic cancer organoids a new approach for label-free drug efficacy analyses by combining high-density microcavity arrays and a high-content imaging system together with AI. The assay does not need any dye and requires less time and data storage and less phototoxicity than traditional Live/dead. Moreover, it can be performed in an automatable high-throughput fashion.

Presentation: Poster

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The multi-cellular integrated human brain (miBrain) to predict, understand, and treat neurodegenerative disease

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The human brain of many individuals is genetically susceptible to neurodegenerative diseases like Alzheimer's disease (AD). However, identifying these individuals, pinpointing the genetic elements that promote pathogenesis and establishing their underlying cellular and molecular mechanisms remain major challenges. These challenges are reflected in the growing patient population and the near absence of therapeutics for AD and related dementias. To address this growing need, we developed a human brain-on-chip (miBrain) from induced pluripotent stem cells that contains all the major cell types and tissues including a blood-brain barrier, electrically active neurons insulated by myelin, and the brain's innate immune cells- microglia. Applying our human brain models has already led to the discovery of two different drugs that reduce cerebrovascular pathology, increase myelination, and memory in aged AD mice (*Nature Med*, 2020 and *Nature*, 2022). We are actively engineering miBrain imaging hardware, and analysis software to enable continuous live data acquisition from the miBrain. Our current miBrain platform consists of an array of 9 mini-microscopes to simultaneously characterize neuronal calcium dynamics in 9 different miBrains. In parallel, we established a panel of live imaging assays to quantify AD pathology (amyloid, tau, synuclein, and inflammation) and subcellular functions (lysosomal pH and size, mitochondrial trafficking, fragmentation, and age, and cell division) in the miBrain. We are multiplexing these assays to permit simultaneous longitudinal monitoring of multiple cellular, sub-cellular, and AD-related phenotypes in the miBrain. We have confirmed that the miBrain can model genetic susceptibility to AD. We are currently expanding beyond genetics to multivariable interactions such as the combined effect of genetics and diet on AD pathogenesis. Using iPSC lines from individuals with available matched postmortem brain tissue and quantitative pathology, we are optimizing the miBrain to model the pathological end-states that are unique to each individual. Through these studies we will establish the miBrain to model the pathological outcomes in each individual's brain and the molecular and cellular events that led up to it. Ultimately, we aim to develop a platform that can predict each person's probability of neurodegenerative disease and identify preventions or treatments prior to clinical onset.

Presentation: Poster

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e-Transmembranes: Bioelectronics and bioengineering synergy to investigate host-microbiome interactions *in vitro*

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The implications of gut microbiome in various aspects of health and disease are a well-established concept in modern biomedicine. Over the past decades, numerous studies have revealed links between host health and microbial activity, spanning from digestion and metabolism to autoimmune disorders, stress and neuroinflammation [1]. However, the exact mechanisms underlying the complex cross-talk between gut microbiota and the host remain a mystery. Conventionally, studies looking at host-microbiome interactions rely on animal models, but translation of such findings into human systems is challenging, mainly due to interspecies differences. Bioengineered models, such as organs-on-chips (OoCs) and human tissue equivalents, represent a highly promising alternative technology, apt for tackling such challenges [2]. Here, we use a novel 3D bioelectronic platform, namely e-Transmembranes [3], to develop a 3D model of the human gut and we implement it in a proof-of-concept study looking at the impact of microbiota on the intestinal barrier integrity. More specifically, we test the effect of different postbiotics on the morphological and functional properties of the barrier, by means of in-line electrical readouts, cross-validated with microscopy assays. We then undertake the same study using live bacteria with known pro-inflammatory, anti-inflammatory or commensal role. Quantification of gut barrier integrity biomarker levels further validates our electrical readouts, showcasing the unparalleled capabilities of e-Transmembranes as in-line sensors of the status and activity of complex biological systems, particularly suited for studying the dynamics of host-microbe crosstalk. Currently, we are working on including in our bioelectronic models more tissue-representative cells (e.g., gut organoids, immune cells, and neurons). We anticipate that our platforms, as well as the tissue engineering strategy and bioelectronic assays established here, will serve as a framework for further studies, including disease modelling, toxicology, and drug screening/development applications.

References

[1] Cryan, J. F. et al. (2019). The microbiota-gut-brain axis. *Physiol Rev 99*, 1877-2013.

[2] Moysidou, C. M. and Owens, R. M. (2021). Advances in modelling the human microbiome-gut-brain axis in vitro. *Biochem Soc Trans 49*, 187-201.

[3] Pitsalidis, C. et al. (2022). Organic electronic transmembrane device for hosting and monitoring 3D cell cultures. *Sci Adv 8*, 4761.

Presentation: Poster

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Exploring the pathogenesis of *Campylobacter jejuni* using a Caco-2 intestinal organ-on-a-chip

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Campylobacter jejuni remains a leading cause of food- and water-borne enteric illness worldwide. Whole genome sequencing of isolates has increased our understanding of how different *C. jejuni* strains are genetically related, their potential reservoirs and carriage of virulence genes, but has been unable to elucidate why some strains are more likely to cause disease and outbreaks than others. Current 2D human cell-based and animal models of pathogenesis have also been unable to fully answer this question although they have indicated that adherence and invasion of the intestinal epithelium are important mechanisms of pathogenesis. We are using a 3D human colon organ-on-a-chip model to study *C. jejuni* pathogenicity in a system that simulates the human gastrointestinal environment. The intestinal organ-chips were seeded with Caco-2 colorectal adenocarcinoma cells and grown for 7-8 days under microfluidic flow and stretch conditions to simulate intestinal physiology. Epithelial barrier function was confirmed by cascade blue dye exclusion and then organ-chips were infected with well characterized *C. jejuni* strains 81-176 (ST-42) or NCTC11168 (ST-15) at a MOI < 1 for up to 6 days (144 hours). Both strains were able to attach and invade the 3D Caco-2 cell structure but did not cause overt epithelial cell toxicity or disrupt the barrier function over the entire exposure period. *C. jejuni* was found dispersed throughout the depth of the 3D epithelial layer as visualized by confocal microscopy, with evidence of apical to basolateral transition. Release of cytokines (TNF-α, IL-6, IL-8 and IL-10) was also assessed. Results from these studies will allow an understanding of the genotype-phenotype relationships in *C. jejuni* in a 3D organ-chip model and will begin to uncover additional insights into the differential pathogenicity of this microorganism.

Presentation: Poster

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Precise control of oxygen in a tumor-on-chip model to study drug resistance

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Tumor-on-chip are promising models to recapitulate *in vitro* the 3D architecture and the physiology of human solid tumor, such as cell-cell and cell-matrix interactions as well as biochemical gradients of drugs and nutrients. To accurately mimic *in vivo* conditions in tumor-on-chip, an important aspect is the reproduction of the gaseous environment. *In vivo* tumor cells experience low oxygen levels (15 mmHg for lung cancer), called "hypoxia", as compared to "physioxia" seen in healthy tissue (40-145 mmHg for lung) [1]. This hypoxic environment is mainly due to the fast proliferation rate of tumor cells along with the creation of abnormal vasculature. Up to date, there are no commercial systems capable to reproduce gradients of oxygen and pH inside microfluidic systems, mimicking not only global hypoxia, but also the fluctuations of local oxygen concentration due to angiogenesis and vessel leakages. We recently developed a new system, called OXALIS (Oxygen ALImentation System), to control the dissolved oxygen level in microfluidic chips with unprecedented performance in terms of response time (200 sec), accuracy (2 mmHg) and liquid flow control accuracy (0.1 µL/min) [2]. We are currently exploiting this precise and fast level of oxygen control to address new biological questions about hypoxia-driven drug resistance. Hypoxia induces enlargement of mitochondria, due to abnormal fusion, which results in apoptosis inhibition and drug resistance [3]. Moreover, drug treatments alter mitochondrial phenotypes. By live imaging, we are conducting a continuous monitoring of mitochondrial shape and size, while oxygen cycles are applied to tumor-on-chip devises containing two populations of lung cancer cells, either resistant or sensitive to the paclitaxel chemotherapy drug. This novel combination of oxygen control and live imaging of mitochondria morpho-dynamics in a tumor-on-chip pave the way to a better understanding of hypoxic cell survival.

References

[1] Mckeown et al. (2014). Defining normoxia, physoxia and hypoxia in tumours. *Br J Radiol 87*, 1035.

[2] Bouquerel et al. (2022). Precise and fast control of the dissolved oxygen levels in tumor-on-chip. *Lab Chip 22*, 4443-4445.

[3] Pahima et al. (2018). Hypoxic-induced truncation of voltage-dependent anion channel 1 is mediated by both asparagine endopeptidase and calpain 1 activities. *Oncotarget 9*, 12835-12841.

Presentation: Poster

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Multi-faceted role of platelets in inflammation and haemostasis in a vessel-on-a-chip model

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Platelets are small, anucleate blood cells whose primary function is in haemostasis at vessel injury sites. Intriguingly, platelets also regulate inflammation. Recent mouse models support a dual role for platelets in promoting inflammation and recruiting neutrophils while simultaneously preventing red blood cell (RBC) leakage (bleeding) at neutrophil transmigration sites. However, conflicting results have been found depending on the organ or model of inflammation studied.

We previously developed a humanized inflammation-on-a-chip model of neutrophil transmigration and extracellular matrix (ECM) infiltration in the Mimetas OrganoPlate [1]. Now, we have extended this model by incorporating platelets and RBCs, allowing us to investigate the role of platelets in inflammation and inflammatory haemostasis.

Unstimulated vessels exhibited very little leakage of small fluorescent dextrans, indicating robust barrier function. RBCs were also retained by unstimulated vessels. Interestingly, permeability to fluorescent dextrans was influenced by matrix composition. Platelet perfusion significantly decreased dextran leakage, confirming a key role for platelets in stabilising non-inflamed vessels.

Inflammatory stimulation of vessels increased dextran permeability and neutrophil transmigration. Bleeding occurred in an ECM-dependent manner. In one ECM, bleeding required inflammation and neutrophil transmigration, whereas in another, inflammation alone induced bleeding. In both matrices, platelets enhanced inflammation, significantly increasing dextran leakage and neutrophil transmigration. Moreover, in both matrices platelets prevented bleeding. To the best of our knowledge, this is the first organ-on-a-chip model of inflammatory haemostasis.

Finally, we studied the role of platelets in another model of leaky vessels, angiogenesis. We induced angiogenesis into the ECM and perfused fluorescent dextrans and RBCs through the newly formed vessels. Bleeding occurred in the absence of inflammation or neutrophils but was ameliorated by platelets.

Together, these data suggest that induction of bleeding depends on the underlying stability of the vessel, which is influenced by ECM composition. This could explain the inconsistent results from different vascular beds in mouse models. Overall, this work

demonstrates the potential of organ-on-a-chip models for modelling interactions *in vitro* that are difficult to investigate *in vivo*.

Reference

[1] Riddle, R. B. et al. (2022). Endothelial inflammation and neutrophil transmigration are modulated by extracellular matrix composition in an inflammation-on-a-chip model. *Sci Rep 12*, 6855.

Presentation: Oral

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Modeling the joint on a chip: A mechanically active microfluidic system to engineer 3D multi-layer osteochondral tissues and investigate osteoarthritis processes to a single cell level

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Osteoarthritis (OA), the most prevalent musculoskeletal disease, is a degenerative disorder mainly affecting load bearing joints. Correlated with mechanical dysregulation, OA leads to cartilage degeneration, to its vascular invasion, and to subchondral bone alterations [1]. No disease modifying OA treatment is presently available, also due to the absence of satisfactory comprehensive models incorporating the subchondral layers. In this work we propose a new Organ-on-Chip (OoC) concept for the easy generation of stacked, directly interfaced 3D multi tissues microconstructs. Leveraging on this technology we pioneer a new mechanically active joint-OoC comprehensive of both a cartilage and a (vascular)subchondral compartment.

Building upon our Cartilage on-chip model [2] we engineered a mechanically active device with a vertical burst valve to pin a suspended fluid so that two subsequent injections can be realized, generating a multi-layer construct. A co-culture joint model was realized with chondrocytes (CHs) for the cartilage layer and a combination of mesenchymal stromal cells (MSCs) and HUVECs for the subchondral compartment. Cells were embedded in an enzymatically formed, metalloproteinase (MMP) sensitive PEG hydrogel [3].

The device was validated demonstrating production feasibility and functioning of the vertical valve concept. Computational simulations adopted to estimate the strain field revealed a strain gradient (ranging from 30% to 0.3%) reminiscent of that of *in vivo* OA joints. Immunofluorescence images of the co-culture demonstrated the possibility of achieving positionally defined complex tissues with a cartilaginous layer interfaced with a (vascular) mineralized tissue. The distinct compression levels applied to cartilaginous microconstructs hosted in the two device compartments induced differences in the expression of OA markers such as PTGS2, C-JUN, and MMP13, and release of the inflammatory cytokine IL8.

We further demonstrated at a single cell level that the osteochondral model is effective in inducing complex CHs subpopulations and possesses an OA-like genetic signature.

Our Joint on-chip model paves the way for the *in vitro* dissection of phenomena such as cartilage degradation and vascular invasion and the testing of innovative OA drug targets and regenerative therapies.

References

[1] Goldring and Goldring (2016). *Nat Rev Rheumatol*.
[2] Occhetta and Mainardi et al. (2019). *Nat Biomed Eng*.
[3] Ehrbar et al. (2007). *Biomacromol*.

Presentation: Poster

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Robustness study of commercial human iPSC-derived cardiomyocytes regarding contractile properties

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Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) serve as ideal human-based cell model for assessing preclinical cardiac risk of new drugs. The combination of hiPSC-CMs and modern cell-based assay systems allows animal-free evaluation of the main cardiac endpoints (electrophysiological properties, calcium handling and contractility) that are addressed during preclinical drug development. Commercial hiPSC-CMs play an important role as stable source for preclinically involved cell-based assays, nevertheless stable lot-to-lot consistency is needed to assess potential hazardous side effects reliably.

Here we demonstrate the lot-to-lot consistency of commercial hiPSC-CMs (iCell Cardiomyocytes^2, FCDI) functionally assessed with the FLEXcyte 96 technology regarding their contractile behavior upon treatment with gold standard compounds nifedipine and sotalol.

Commercial hiPSC-CMs were cultured on FLEXcyte plates 6 days before compound treatment. A control measurement was per-

formed before compound addition to evaluate general cardiomyocyte contraction behavior and compared among different cell lots regarding two parameters – contraction amplitude and beat rate. Cells were then treated acutely with two different concentrations of nifedipine and sotalol. Changes in amplitude or duration as well as beat rate were analyzed and compared among different cell lots.

The results show comparable contraction amplitudes and beat rates of the untreated cell lots demonstrating a stable performance of hiPSC-CMs before compound treatment.

The pharmacological comparison of ten lots with sotalol and nifedipine also shows comparable results with non-significant fluctuations.

The pharmacological assessment of ten different commercially available hiPSC-CM lots proved that lot-to-lot variations do not alter contractility related data analyzed with the FLEXcyte 96.

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Automated organ-on-chips for reducing intralaboratory cell culture variability

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Microphysiological systems (MPS), and especially Organs-on-Chips (OoCs), have emerged as a potential revolution in the field of pharmaceutical development and diseases study. The legislative bodies are even starting to revise their text to accommodate for these technologies. However, as the complexity of OoCs increases to fully address the current and future scientific needs, these technologies still struggle to move from the laboratory into real-life medical care for citizens all around the world.

There are still some significant challenges that need to be overcome to fully enable this transition. The lack of trained technicians on microfluidic operations, the high exigence from the industry (high throughput screening and high reproducibility) and the integration with their current technologies and readouts constitute some of these roadblocks to remove.

To address these needs, we present a study using our OoC devices (compatible with ANSI SLAS 4-2004 (R2012) (formerly recognized as ANSI/SBS 4-2004) norms) grown in an automated cell culture platform (Biomek 7, Beckman Coulter) in the presence of primary hippocampal neurons of rodents. This study compares the reproducibility between cultures performed by an experienced technician and an automated cell culture robot. The time saved by using an automated cell culture platform is also monitored. Several reproducibility endpoints are evaluated such as media replacement efficiency, cell density and homogeneity at seeding and after two weeks of culture, axonal growth measurements, neurotransmitters dosage, and fluorescent profiles for various cellular markers.

This study demonstrates the high level of efficiency and reproducibility achievable when using OoC platforms compatible with automatic cell culture robots. This quest to reduce intralaboratory variability is essential to reach sufficient performance levels for regulatory submissions for clinical trial of new drugs. Moreover, we believe that designing OoCs with ANSI and SBS norms in mind, thus being compatible with automated culture and data acquisition platforms, will be a crucial step towards fulfilling the needs of industrial companies in terms of scaling and efficiency.

Presentation: Poster

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Functional skin-on-a-chip, a relevant *in-vitro* platform to replace animal models in drug and cosmetic development

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Topical drugs and cosmetic developments are limited by *in-vitro* models, which are not always relevant either because they use rodent cells, banned in the field of cosmetic, or because the cocultures of skin and neurons do not fully recapitulate the anatomical structure. To address these limitations, we developed microfluidics devices using human cells (human iPSC-derived cells and primary cells) to reproduce i/ skin compartment with keratinocytes and nerve endings and ii/ spinal cord compartment with neuronal cell nucleus. We developed PDMS pumpless compartmentalized microfluidic devices allowing a fluidic isolation while allowing the physical connection between neurons' axons and skin cells. Viability, maturation, and functionality have been successfully proved. The different chips we developed might be used either for high throughput screening or to study mechanism of action or pathophysiology.

Furthermore, our devices can be coupled to microelectrode array technology allowing electrical signals recoding of soma after axonal stimulation, leading to innovative readout for the dermo-cosmetic research.

Presentation: Poster

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Versatile organ-on-a-chip model allowing air-liquid interface and blood barrier

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These days, a major challenge for tissue engineering is the creation of versatile and functional 3D tissue. However, the construction of thicker tissue and the culture of organoids require an adequate supply in oxygen and essential nutrients through blood vessels. Cell culture inserts may represent relevant models to achieve co-culture on both sides of a membrane, however they may have limitations specially to create blood vessels structures close to the physiology.

We developed a microfluidic device consisting of a large channel which might be colonized by endothelial cells, in the middle of which an open well, separated from the channel by a transparent membrane, in which organoids or epithelial cells might me cultivated.

We characterized the device from microfluidic and biological aspects. This microfluidic device allows the application of a shear stress in order to ensure the functionalization of endothelial cells and to mimic the blood flow. The integrity of the membrane has also been characterized by TEER and Dextran transport evaluation.

In addition, thanks to the open well, an air-liquid interface might be performed.

The versatility of our devices allows their evolution for example by connecting other compartments to add other cell types.

Presentation: Poster

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Human gastric organoids reveal *Helicobacter pylori* tropism to differentiated pit cells dependent on chemotaxis

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We use adult stem cell derived organoids to study infection, epithelial innate immunity and cancer in the human gastrointestinal epithelium. The human gastric epithelium forms highly organized gland structures with different subtypes of cells. The carcinogenic bacterium *Helicobacter pylori* infects gastric cells via attachment and subsequent translocation of its virulence factor CagA, but the possible host cell tropism of *H. pylori* is currently unknown. Single cell sequencing of 3D organoids and 2D monolayers derived from organoids demonstrate that the first mostly contains cells from the gastric gland region, while the latter contains pit cells and especially a population of highly differentiated pit cells. This population is absent from 3D organoids under standard growth conditions but can be generated using directed differentiation. ScRNA-seq of infected cells demonstrates, that *H. pylori* preferentially binds to this subpopulation of differentiated pit cells expressing PSCA, GKN1 and GKN2. Compared to gland cells, the differentiated pit cells mount a lower innate immune response to the bacteria. The binding preference depends on chemotaxis of the bacteria towards host cell metabolites. Through this very sensitive chemotaxis, evolution has shaped a system, in which *H. pylori* target those cells that are not only mounting the lowest immune response but that are also replaced most frequently in the epithelium.

Presentation: Poster

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Human gastrointestinal organoids show patterning of innate immune signalling along the cephalocaudal axis

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In the gastrointestinal tract, the epithelial lining acts as physical and immunological barrier between the microorganisms of the gut and the body. The epithelial cells can sense microorganisms, which activates innate immune signaling pathways and leads to an inflammatory response. We generated a biobank of human and murine organoids covering 6 sites from stomach to colon. RNA-sequencing showed that the tissue identity is conserved in the adult stem cells. Moreover, components of the epithelial innate immune sensing, such as toll like receptors, are part of the tissue identity and highly organized along the gastrointestinal tract. This organization is for a large part determined by developmental processes rather than by environmental factors.

Presentation: Poster

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Towards a dynamic *in vitro* model of the intestine using smart hydrogels

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Intestinal peristalsis plays a crucial role in digestion, nutrient absorption, and susceptibility to diseases. Mechanically active intestinal models capable of undergoing peristalsis are very attractive because they reduce the dependence on testing in animal models and mimic intestinal physiology [1]. However, physiologically relevant peristaltic contractions are difficult to recreate *in vitro* due to practical difficulties in achieving force control and periodicity during compression of soft substrates. In this work, we explore possibilities of achieving gut motility *in vitro*, in a non-contact manner, using light as a trigger.

To mimic intestinal muscles that cause a peristaltic contraction, we generate light-responsive hydrogel micro-particles using three different techniques- microfluidics, electrospinning, and atom-transfer radical polymerization. The first proof-of-concept is a trans-well setup, containing the micro-particles in a hydrogel representing the basement membrane of the intestine, where the intestinal epithelium and mucus-producing cells are represented by Caco-2 cells and HT-29 MTX respectively. Different ratios of these cells are co-cultured to mimic different parts of the intestine such as the colon and the small intestine. Cell differentiation, accompanied by the formation of micro-villi, is studied by electron microscopy. Monolayer barrier properties are assessed through trans-epithelial electrical resistance (TEER) measurements and permeability response to different-sized molecules and pathological proteins of clinical relevance such as alpha-synuclein is evaluated.

We show successful fabrication of micro particles via electrospinning and confirm our observations with electron microscopy. Culturing cells on basement membrane mimicking hydrogel, we observe altered micro-villi formation as well as mucus secretion *in vitro*, when compared to a stiff substrate such as poly(ethylene terephthalate) that is often used in intestinal models. We also demonstrate physiologically relevant barrier integrity of cellular monolayers after differentiation on such substrates, which are confirmed by TEER and barrier permeability evaluation using dyes of different molecular weights.

Thus, we aim at designing a modular platform where mechanical cues can be user-defined, and which has great potential to investigate intestinal permeability in a physiologically relevant manner to gain insights into the contribution of gut motility to human health.

Reference

[1] Jalali-Firoozinezhad et al. (2019). *Nat Biomed Eng*.

Presentation: Poster

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Developing a scaffold-based model of neurodegeneration for drug discovery using 3D bioprinting

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Alzheimer's disease (AD) and Parkinson's disease (PD) are the most prevalent neurodegenerative diseases and are expected to increase in line with global lifespan for the foreseeable future [1]. A major component of the failure to bring novel drugs for neurodegenerative diseases to market is the translational gap between *in vitro*, animal, and human studies. Advances in the development of microphysiological systems has the potential to bridge this gap. However, the complexity of microphysiological systems can lead to high heterogeneity and labour-intensive development. Consequently, some may consider the complexity of microphysiological systems to be incompatible with screening applications in drug discovery, which require higher throughput and low batch-to-batch variability.

Using 3D bioprinting, we aim to develop scaffold-based models of neurodegenerative diseases for drug screening using human induced pluripotent stem cells (iPSCs), increasing throughput, and reducing heterogeneity. Using the RASTRUM® 3D bioprinter, we have developed a scaffold-based model of the brain in 96- and 394-well plate formats using iPSC-derived neural cell types (glutamatergic neurons, GABAergic neurons, astrocytes and microglia) and high-resolution printing [2]. The quad-culture is suspended in a hydrogel of custom matrix proteins modulated to mimic brain biomechanics to encourage neural cell binding and growth.

This model is being adapted to mimic the pathophysiology of AD and PD using mutant iPSC lines, and with hydrogel patterning and composition, these models will map the structure of relevant brain areas. This will be combined with changes in the hydrogel matrix to replicate changes in biomechanics observed in aging [3]. In summary, this model aims to revolutionise modelling of neurodegenerative disease for drug discovery applications through balancing complexity and convenience.

References

[1] Peplow, P. V. et al. (2022). Prevalence, needs, strategies, and risk factors for neurodegenerative diseases. In P. V. Peplow et al. (eds), *Neurodegenerative Diseases Biomarkers: Towards Translating Research to Clinical Practice* (3-8). Springer US.
[2] Utama, R. H. et al. (2021). A covalently crosslinked ink for multimaterials drop-on-demand 3D bioprinting of 3D cell cultures. *Macromol Biosci 21*, 2100125.
[3] Guo, J. et al. (2019). Brain maturation is associated with increasing tissue stiffness and decreasing tissue fluidity. *Acta Biomater 99*, 433-442.

Presentation: Poster

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Standardized patient-derived rectal organoids predict clinical efficacy of CFTR modulator in a patient with the rare 1677delTA/R334W genotype

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Cystic fibrosis (CF) is the most common genetic disorder, with an incidence of approximately 1:2500 live births and is characterized by a chronic damage of all epithelial organs. It is caused by a large variety of mutations on the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In the past ten years, CFTR modulators have revolutionized CF standard of care, leading to significant improvements of most of the symptoms and ameliorated quality of life for many patients. However, despite an increase overtime of the list of variants eligible for CFTR modulators, patients having rare and poorly characterized mutations that are not listed in the official guidelines still have no access to these treatments. In this context, *in vitro* analysis using patient-derived rectal organoids can be used as a tool to predict patient responses to treatments in a personalized manner and to guide tailored therapy. We report the case of a CF individual with a specific combination of mutations (1677delTA/R334W), that thanks to the prediction based on the organoids assay received access to the CF modulator Ivacaftor. The dramatic clinical improvement of this patient has demonstrated that intestinal organoids are a key technological advance to better understand the impact of different treatments on the organoid epithelia, enabling a more precise tailoring of therapies, and thus supporting treatment decisions for complex CF phenotypes.

Presentation: Oral

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Assessing efficacy of combination therapies in human colorectal cancer organoids using a standardized screening workflow

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Precision medicine for cancer patients promises the tailoring of targeted therapies to specific genetic alterations. Currently, alterations in 43 oncogenes can be targeted based on Level 1 clinical evidence. Still, the majority of cancer patients lack efficient targeted therapy options with lasting benefits.

Ex vivo assays, such as tumor tissue explants, hold the promise to directly measure the impact of anticancer compounds and their combinations. However, a significant challenge for *ex vivo* drug testing lies in the efficient establishment of fresh primary cell cultures for testing, within a clinically actionable timeframe, and in the available tumor volume. To this end, patient-derived organoids (PDOs) have been proposed as viable and efficient alternatives for *ex vivo* testing. PDOs show long-term expansion potential while retaining tumor histopathology as well as cancer gene mutations. We have shown how homogenous reproducible PDOs based on Gri3D® hydrogel microwell arrays could be generated for high-throughput drug testing of single and combination therapies.

Here we show on human colorectal cancer organoids the ability to perform dose-response analysis of multiple anti-cancer drugs, which can be used to guide the selection of optimal drug selection for a patient. In addition, we demonstrate how a combination of anti-cancer drugs could enhance efficacy compared to single-therapy approaches. By targeting pathways in a synergistic or additive manner, a lower therapeutic dosage of each individual drug is required, potentially also reducing toxic side effects.

Presentation: Poster

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Multi-niche human bone marrow-on-a-chip for plasma cell survival and differentiation

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Antibody secreting cells (ASCs) are terminally differentiated cells key to the humoral immune response. They are responsible for producing and secreting antigen-specific immunoglobulin antibodies to neutralize and help eliminate pathogens. ASCs differentiate from activated B cells in lymph nodes and transiently circulate in the blood before migrating to the bone marrow (BM) and eventually maturing to long-lived plasma cells (LLPCs). Despite decades of immunological discoveries, the differentiation and maturation programs of early circulating ASCs to LLPCs remain elusive [1]. The main constraints are challenges in successfully culturing plasma cells *in vitro* and lack of an *ex vivo* human B cell supportive BM niche that allows for maturation of plasma cells. To circumvent these limitations, we have bioengineered a BM-chip that incorporates the endosteal, central marrow, and perivascular niches of the human BM where LLPCs differentiate and reside [2]. Herein, we studied how the multi-niche BM-chip may support the survival, differentiation, and niche-specific homeostasis of B cells and plasma cells.

The microfluidic BM-chip consists of a human mesenchymal stromal cell (MSC)-derived mineralized endosteal layer surface that differentiates over 21 days. Then human endothelial cells and MSCs are loaded in a collagen-fibrin hydrogel atop the endosteal layer, forming a 3D microvascular network representing the central marrow and perivascular niches. Confocal imaging and analysis of cytokine secretion reveals that the BM-chip expresses key components of the BM niche essential for ASC retention and survival including CXC12, hyaluronan, and IL6. Next, CD19+ or CD138+ B cells enriched from peripheral blood or B cell organoids derived from human tonsil samples or PBMCs were perfused into the BM-chip through the microvascular network. After 7 days of culture in the BM-chip, the cells took on a CD19loCD27+CD38++ phenotype compared to day 0, indicating potential differentiation towards ASCs. Our results indicate that this BM-chip has the potential to support plasma-like cells *ex vivo* and provides a novel system for studying the BM microenvironment required for plasma cell maturation and maintenance.

References

[1] Nguyen, D. C. (2018). *Nat Com*.
[2] Nelson, M. R. (2021). *Biomat*.

Presentation: Oral

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An inflamed alveolus model on a breathing lung-on-chip for investigation of human anti-inflammatory drug response

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Pre-clinical testing of inhaled drugs to study pulmonary diseases or estimate human drug response still relies on animal data due to the lack of predictive and validated alternative *in vitro* models.

In this study, we established an inflamed alveolus model on a breathing lung-on-chip to mimic (patho-) physiological processes and predict human anti-inflammatory drug response. The lung-on-chip technology provides insights into the relevance of cellular diversity, alveolar structure, dynamic environment and aerosol exposure in a complex *in vitro* model [1]. To reconstitute the human-air-blood-barrier in the lung-on-chip, we cultured the tight human alveolar epithelial cell line Arlo [2]. To enhance physiological relevance, the introduction of important *in vivo* parameters was investigated, including the presence of alveolar macrophage surrogates, the application of 3D stretching to emulate breathing motion in the alveoli and the nebulization of anti-inflammatory treatment.

The application of lung-on-chips in the context of lung inflammation was assessed by using two different pro-inflammatory stimuli: bacterial lipopolysaccharide (LPS) and a combination of pro-inflammatory cytokines TNFα and IFNγ. Read-outs were cytokine release, measured via bead-based FACS assay, and the measurement of barrier integrity via TEER or apparent permeation of the small molecule fluorescein. For both pro-inflammatory stimuli, the presence of macrophages as the immune system component in the deep lung was crucial to properly simulate the inflammatory response. Using a synergistic combination of TNFα and IFNγ, barrier breakdown and protection were most pronounced in breathing conditions. For both pro-inflammatory stimuli, the protective effect of the anti-inflammatory treatment of Budesonide as an exemplary inhaled anti-inflammatory drug was demonstrated.

These findings may represent an important next step towards more predictive models for human drug development as alternatives to ethically challenging animal-based studies.

References

[1] Sengupta, A., Roldan, N. et al. (2022). A new immortalized human alveolar epithelial cell model to study lung injury and toxicity on a breathing lung-on-chip system. *Front Toxicol 4*, 840606. doi:10.3389/ftox.2022.840606

[2] Carius, P., Jungmann, A. et al. (2023). A monoclonal human alveolar epithelial cell line ("Arlo") with pronounced barrier function for studying drug permeability and viral infections. *Adv Sci 10*, e2207301. doi:10.1002/advs.202207301

Presentation: Poster

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A lentiviral reporter system for live imaging of cell differentiation and mucus production in human lung organoids

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The majority of animal models used to study the human lung, have, up to date, failed to reproduce the characteristics of the human lung environment, in an accurate and reliable way and in accordance with the animal welfare. Ultimately, researchers are looking for a sustainable pre-clinical model that can help screen drug compounds in a high-throughput manner (Sprott et al., 2020), in order to increase the likelihood of success in human clinical trials, saving time, resources and sparing animal suffering in the process.

Therefore, this study intends to develop a flexible lentivirus-based reporter system to tackle differentiation and mucus production in human organoids. We designed fluorescently tagged lentiviral constructs for live imaging of cell specific promotor activity of basal cells, ciliated cells, club cells and secretory cells. Primary bronchial epithelial cells for organoid formation were derived from healthy donors and/or from patients with respiratory co-morbidities. Time-lapse fluorescent imaging was established to track changes in cell populations during 21 days of organoid development and confocal imaging was used for representative images at given time points. Our results demonstrate that these organoids developed from basal stem cells start expressing differentiation markers for ciliated and secretory cells over time, yet club cells are not present in our model. To evaluate the robustness of our model, we stimulated the organoids with IL-13 and/or Notch-2 inhibitors. As reported in previous studies (Atherton et al., 2003; Danahay et al., 2015), IL-13 stimulation induces a significant reduction of ciliated cells paired with goblet cell hyperplasia, as evidenced by the increase in secretory activity and a decrease of ciliated cells, while NOTCH-2 inhibition rescued this phenotype restoring the ciliated cell populations.

This study presents a robust and reproducible live imaging system for high throughput screening of drugs and novel compounds in human lung organoids. The ease of infection of basal stem cells, allied with developing organoids directly from patient samples, is a major step towards personalized medicine. Likewise, the ver-

satility of lentiviral manipulation means that our reporter model might have broader applications beyond the human lung and could be established for other organs and pertinent diseases.

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Neuro-muscular system (NMS) for botulinum neurotoxin assays to replace animal testing: Readouts, applications, and regulatory qualification standards

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The Mouse bioassays (mice lethality assays and/or mouse protection assays) are standard testing methods that meet FDA's regulatory requirements to quantitate the biological activity of botulinum neurotoxins (BoNTs). They are used for assessing the drug product potency, stability, or safety of botulinum-based drug products, or botulinal medical countermeasure products (BMCM), such as antidote drugs, botulinum toxoids, and antitoxins, etc. to meet the regulatory submissions and/or for the GMP product release. Mouse bioassays are also used to qualitatively confirm the presence of toxins during foodborne outbreak investigations in public health laboratories for informed decision making and meet food safety regulations. A replacement to mouse bioassays for regulatory use should utilize endpoint(s) that holistically assess all the three major cell binding, membrane translocation, and enzymatic activity processes of BoNTs, and the endpoint readout(s) should also be specific to their mechanism of action (MOA). *In-vitro* neuromuscular junction (NMJ) based microphysiological systems are potential platforms to reduce or replace the current mouse bioassays for regulatory evaluation of BoNT based drugs and BMCM products. Such microscale NMJ system can enable us to detect or measure the biological activity of BoNTs as they inhibit the synaptic transmission processes of the NMJ to cause paralysis. We will discuss the critical performance requirements and validation criteria needed for BoNT assays; merits of different readouts and microfabrication levels for preclinical research, GMP-manufacturing and other regulatory applications.

Presentation: Oral

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Reproducible production of bioengineered homogenous hPSC-derived organoids on a microplate

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Human pluripotent stem cells (hPSCs) derived organoids have been increasingly recognized as a human physiologically relevant *in-vitro* model system for disease's mechanistic studies and preclinical drug evaluation, as these organoids may carry patient-specific phenotypes and contain tissue-specific cell types across several germ layers. Currently, widely-adopted hPSC organoid production methods include the Matrigel dome culture method for endoderm organoids and the suspension culture method for ectoderm organoids. However, the hPSC organoids generated by these methods suffer from several drawbacks, including (i) large inter-organoid variability in their morphology, size, and maturity hampers quantitative bioanalysis; (ii) low reproducibility of organoid formation in varied batches affects wide applications of organoid in the industry; (iii) non-deterministic location of organoids make it difficult for the same-single-organoid monitoring and analysis.

In this study, we demonstrate several bioengineered hPSC organoid production methods, including micropatterning, microcavity array, and micromolding techniques. hPSC-derived liver and brain organoids are produced using these bioengineering methods and compared to those formed by conventional methods in morphogenesis, maturity, and reproducibility. The bioengineering methods permit the production of more than 8000 organoids in a 48-well plate. Quantitative image and qPCR analysis indicate that bioengineering methods can significantly improve intra-batch homogeneity and inter-batch reproducibility. Specifically, the coefficient of variation of gene expression and organoid size can be controlled within 20%. Additionally, the bioengineering method allows a deterministic location of organoid formation that facilitates *in-situ* long-term same-organoid imaging and analysis. As a proof-of-concept, we demonstrate the bioengineered hPSC organoids to evaluate drug toxicity. We expect these bioengineered hPSC organoids to find wide applications in biomedical research and drug discovery.

This study was supported by grants from the National Natural Science Foundation of China (No. 82272173).

Thanks for the technical support from the Innovations in stem cell and organoids project (ISCO).

References

[1] Xu, X. et al. (2022). *Front Bioeng Biotechnol 10*, 937595. doi:10.3389/fbioe.2022.937595

[2] Jiang, S. et al. (2023). *Biofabrication 15*, 015006. doi:10.1088/1758-5090/ac933c

[3] Lancaster, M. A. et al. (2013). *Nature 501*, 373-379. doi:10.1038/nature12517

Presentation: Oral

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Culture medium study for construction of the cardiotoxicity evaluation system via hepatic metabolism

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Introduction: Cardiotoxicity, including arrhythmogenesis is a cause of drug withdrawal from the market. Therefore, candidate compounds, that cause cardiotoxicity, should be identified at an early stage in drug development. Some drugs withdrawn from the market due to cardiotoxicity exhibit toxicity via hepatic metabolism and drug interactions. This cardiotoxicity cannot be identified by an evaluation system using only cardiomyocytes. In addition, extrapolation to humans is not sufficient due to species differences in animal experiments. Hence, a new evaluation system with high extrapolation to clinical data were required. We aim to construct an evaluation system for cardiotoxicity via hepatic metabolism by co-culturing human hepatocytes and engineered heart tissue (EHT) using a stirrer-based circulation pump microphysiological system (MPS), which developed by Kimura et al., Tokai University. This MPS has an extremely small stirrer installed in the microchannel between the wells, allowing medium perfusion. We assume a co-culture of human preserved hepatocytes (cryoheps) and human-iPS cardiomyocyte-derived EHT (hiPS-CM-EHT) as the cardiotoxicity evaluation system via hepatic metabolism using the MPS. Therefore, co-culture medium for each cells to function sufficiently were examined.

Methods: Cryoheps and hiPS-CM-EHT were cultured separately in medium A, B, or C. Expression levels of major CYPs (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) in cryoheps was measured using quantitative PCR. Heart rate, contraction distance, contraction velocity, and relaxation velocity in hiPS-CM-EHT were measured.

Results and conclusion: Expression of CYPs other than CYP3A4 of cryoheps in all media were at the same level as those of human liver. CYP3A4 expression in medium A was at the same level as that in human liver. Whereas CYP3A4 expression in medium B or C also increased to levels similar to the human liver because of oxygen supply improvement. The contraction distance of hiPS-CM-EHT in medium A or B was longer than that in medium C. However, the contraction distance in medium A was shortened at 72 hours. Thus, we consider medium B to be a potential medium candidate for co-culture of cryoheps and hiPS-CM-EHT.

Presentation: Poster

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Integration of deep learning assisted high-content screening and deep tissue-phenotyping to identify cardioprotective compounds in dilated cardiomyopathy

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With a prevalence of 4:10,000, dilated cardiomyopathy (DCM) is the most common hereditary heart disease, characterized by dilatation of one or both ventricles and impaired systolic and diastolic function. Numerous mutations have been identified to cause DCM. TITIN-truncating variants (TTNtv) are commonly observed in 20% of the genetic DCM cases. Progress in drug discovery for DCM has been hampered by the lack of robust high-throughput/high-content analysis pipelines of disease-specific cell and tissue platforms for deep structural and functional phenotyping.

To address this challenge, we have developed a two-tiered human induced pluripotent stem cell (iPSC)-based screening approach, comprised of high-content cardiomyocyte imaging with artificial intelligence (AI)-driven image and data analyses as well as deep tissue phenotyping in engineered heart muscle (EHM) models. As a DCM use case, we have developed several isogenic human iPSC-lines with and without a previously reported and pathologically relevant A-band-TTNtv mutation (c.70692_70693insAT; p.T23565SfsX5) [1-3].

Using fixed weights from a convolutional deep neural network (EfficientNet) trained on ImageNet, we generated unbiased deep embeddings from each 2D cell model and applied these to train machine learning (ML) models to detect morphological disease phenotypes. Our ML model is able to confidently separate healthy controls from TTNtv cells with high accuracy (> 93%), specificity (WT > 79%) across different batches/plate layouts and generate concentration-response curves, demonstrating platform robustness and sensitivity. Building upon this work, we will present our latest efforts applying the platform technology for a chemogenomic screening campaign to identify novel cardioprotective drugs using a library of 7,100 bioactive compounds and validation assays, including measuring functional changes in EHMs.

The phenotypic profiling platform, as presented here, can be readily adapted to other disease-relevant cell types and pathologies. Our results demonstrate the power of combining iPSC-CMs with cytological profiling and deep learning as well as tissue-level phenotyping to accelerate drug discovery for patients with DCM.

References

[1] Gerull, B. et al. (2002). *Nat Genet 30*, 201-204. doi:10.1038/ng815
[2] Gramlich, M. et al. (2015). *EMBO Mol Med* 7, 562-576. doi: 10.15252/emmm.201505047
[3] Fomin, A. et al. (2021). Sci Translat Med 13, eabd3079. doi:10.1126/scitranslmed.abd3079

Presentation: Oral

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Miniaturized joint tissues and living microfluidics to study cartilage degenerative diseases (MINI-JOINT)

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The prevalence of joint inflammatory diseases, such as osteoarthritis (OA) of the knee is ever-increasing. With high associated societal costs and loss of quality of life, in 2019, its incidence was estimated at 250 million people [1]. There is currently no cure for OA. Screening techniques have failed to bring novel potential curative treatments closer to the clinics. Novel and better models are needed. This project focuses on the development of a novel *in-vitro* model to better represent the healthy and diseased state of the knee joint, we aim to bring a cure closer to patients.

We present a bioreactor platform that contains four engineered tissues divided in two independently perfusable chambers. The platform contains a chamber for the perfusion of trabecular bone, while an osteochondral interface layer forms a physical barrier to the second perfusable chamber, which contains engineered articular cartilage and synovial lining. The trabecular bone scaffold was printed via the novel Volumetric Bioprinting technique (VBP) to better represent trabecular architecture [2]. The cartilage/synovium compartment is separated from the bone compartment by a layer of engineered osteochondral interface, also referred to as calcified cartilage. In the native joint, this tissue restricts diffusion between cartilage and bone. The cartilage and synovium compartment are fabricated using custom-made hydrogels and human donor material [3]. Furthermore, to test the potential of the platform to replicate the early onset of cartilage inflammation and the effects it has on joint remodeling, we have developed an inflammation model using a THP-1 cell line. These cells are differentiated into macrophages type 1 (M1) and the supernatant is perfused through the cartilage compartment. Aiming to trigger an OA-like response and observe cartilage deterioration.

The model has been successfully perfused long-term without leakage in any of the interfaces. The MINI-JOINT aims to deliver a platform where to assess the progression of knee OA. We believe that this project brings a more representative *in-vitro* model a step closer to better screening of OA drug candidates.

References

[1] Hunter, D. J. (2019). *The Lancet*.
[2] Bernal, P. N. et al. (2022). *Adv Mater*.
[3] Levato, R. et al. (2017). *Acta Biomater*.

Presentation: Poster

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iPSCs-derived microphysiological system for the study of amyotrophic lateral sclerosis *in vitro*

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Understanding the complex communication between different cell populations and their interaction with the microenvironment is fundamental in neuroscience research. Due to the lack of animal models capable of faithfully reproducing the physio-pathological mechanisms of many human diseases, the development of appropriate *in vitro* approaches and tools, for selectively analyzing and probing specific cells and cell portions (e.g., axons and cell bodies in neurons) has become crucial in this direction. On one hand, the rising technology of organ-on-a-chip offers the possibility to overcome these problems, replicating key units of living organs and organisms [1]. On the other hand, the discovery of human induced pluripotent stem cells (hiPSCs) opened new areas for precision medicine, especially for neurodegenerative disorders, where the number of patients increases while the successful rate in drug discovery remains worryingly low. During the past decades, many platforms have been proposed, focusing the attention on the neuromuscular junction (NMJ), damaged in these disorders and in amyotrophic lateral sclerosis (ALS) [2]. The NMJ is a specialized region composed of presynaptic lower motor neuron (MNs), postsynaptic muscle myofiber and terminal Schwann cells (tSCs), involved in the control of vital body processes. Most of the developed platforms focus on the interaction between MNs and muscle cells since. To date, protocols to generate pure populations of tSCs are not available. Recently, we developed a microfluidic device with three different perfusable compartments interconnected through a series of narrow microchannels in which different cell populations can be hosted [3]. Here, we differentiated on-chip hiPSC, from both healthy and ALS patient donors, towards MNs and SCs and co-cultured them to investigate possible interactions, such as axonal elongation across the microchannels in the presence of chemical cues (BDNF or CNTF) or other cell types (iPSCs-derived SCs). This platform, with the implementation of iPSCs-derived muscle cells, will be useful to study the dysfunctions that occur in the NMJ of patients affected by ALS.

References

[1] Bhatia, S. N. and Ingber, D. E. (2014). *Nat Biotechnol*.
[2] Verma, S. et al. (2022). *Mol Neurobiol*.
[3] De Vitis, E. et al. (2021). *Sci Rep*.

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iPSC-derived brain endothelial microvessels in a standardized multi-chip format as 3D human blood-brain barrier model for drug permeability screens

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Optimizing drug candidates for blood-brain barrier (BBB) penetration remains one of the key challenges in the field of drug discovery to finally target brain disorders including neurodegenerative diseases. It has been difficult to establish state-of-the-art stem cell derived *in vitro* models that mimic physiological barrier properties including a 3D microvasculature in a format that is scalable to screen drugs for BBB penetration. To address this challenge, we established human induced pluripotent stem cell (iPSC)-derived brain endothelial microvessels in a standardized and scalable multi-chip format [1]. In our process, iPSC-derived brain microvascular endothelial cells (BMECs) were matured by primary cell conditioned media that can be produced in larger quantities to facilitate a standardized upscaling. After 10 days of culturing, perfused self-organized microvessels show typical BBB endothelial protein expression, tight junctions, and polarized localization of efflux transporter. Microvessels exhibited physiological relevant trans-endothelial electrical resistance (TEER), were leak-tight for 10 kDa dextran-Alexa 647 and strongly limited the permeability of sodium fluorescein (NaF). Permeability tests with known reference compounds confirmed the suitability of our model as platform to identify potential BBB penetrating anti-inflammatory drugs. Finally, our presented model recapitulates physiological properties and allows rapid screening of BBB permeable anti-inflammatory compounds that has been suggested as promising substances to cure so far untreatable neurodegenerative diseases.

Reference

[1] Fengler, S., Kurkowsky, B., Kaushalya, S. K. et al. (2022). Human iPSC-derived brain endothelial microvessels in a multi-well format enable permeability screens of anti-inflammatory drugs. *Biomaterials 286*, 121525. doi:10.1016/j.biomaterials.2022.121525

Presentation: Oral

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Identifying a common endothelial medium to connect organs-on-chips for CAR-T safety testing

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Introduction: CAR-T cells as immunotherapy show promising results to treat hematologic malignancies as well as solid tumours, but in rare cases can also lead to severe adverse events like neurotoxicity and cytokine release syndrome (CRS) [1]. We are developing a multi-organ-on-chip model to recapitulate both the interaction of CAR-T cells and tumour cells, as well as the potential downstream inflammatory effects on vascular endothelium. The model is currently limited due to lack of medium compatibility between parts of the system. In this study, we tried to optimize the medium composition of the two main models (tumour-on-chip and vessel-on-chip) to eventually test for CAR-T-mediated CRS.

Methods: HiPSC derived endothelial cells (hiPSC-ECs) were seeded in 6-well plates for medium compatibility testing with X-VIVO-15 hematopoietic medium. The cells were grown to confluency, treated overnight with X-VIVO-15 with different supplements, and fixated. Analysis was done by ICAM-1 expression and F-actin staining to visualize monolayer integrity. Tests were repeated in polydimethylsiloxane (PDMS) vessels-on-chip, with viscous finger patterned collagen-1 lumens [2]. These lumens were uniformly seeded with hiPSC-ECs ($5 \cdot 10^6$ cells/ml) [3]. As a proof-of-concept for medium compatibility, cytokine-rich X-VIVO-15 medium was obtained from the tumour-on-chip model, which included antigen-specific CAR-T cells, and used to stimulate the vessels-on-chip overnight.

Results and discussion: 2D experiments showed that X-VIVO-15 did not lead to increased ICAM-1 expression on hiPSC-ECs but did lead to loss of the cell monolayer. This loss could be prevented by supplementing the medium with human-derived plasma, VEGF, and bFGF. On-chip experiments confirmed these findings, meaning that medium from the tumour-on-chip model can be used to stimulate the vessel-on-chip model. First results of supplemented X-VIVO-15 medium of the tumour-on-chip show that the monolayer of the vessel-on-chip stays intact, but ICAM-1 expression is increased if the CAR-T related cytokines in the medium are elevated.

Outlook: Establishing medium compatibility of the two systems is an important milestone in this collaborative project. Experiments towards a connected tumour-on-chip/vessel-on-chip are ongoing. The vessels-on-chips will also be perfused with human whole blood to see differences in intravascular coagulation.

References

[1] doi:10.1016/j.blre.2018.11.002
[2] doi:10.1063/1.5090986
[3] doi:10.1016/j.stemcr.2018.03.012

Presentation: Poster

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Utilization of multicellular liver-on-chip to study non-alcohol-related fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease, affecting nearly one-third of the global population. To study NAFLD, current experimental models e.g., animals and 2D cell cultures, pose various limitations including species-specific differences and lack of complexity. Here, I employ the innovative, human-based organ-on-chip system to overcome these limitations and explore lifestyle-based NAFLD interventions. Overcoming the limitations of current liver model systems with the liver-on-chip, I aim to study the effect of medicinal-implied nutrients ("nutriceuticals") to prevent or reverse NAFLD and the underlying molecular mechanism.

Method: We have recently established a hepatocyte-on-chip model utilizing healthy control-derived human induced pluripotent stem cells (hiPSCs) and a human hepatocyte cell line in our lab. Here, I present data on the development and characterization of a NAFLD-on-chip. Characterization of the NAFLD-on-chip includes steatohepatitis readouts, e.g., lipid accumulation and detection of very-low-density lipoprotein and inflammatory cytokine excretion.

Results: Preliminary data reveals that the hepatocyte-on-a-chip models available in our lab exhibit important features of normal human hepatocyte physiology (e.g., expression of transport proteins and albumin production) and features often not observed in "static" cell culturing models (e.g., lipoprotein excretion). Additionally, we identified the conditions to induce NAFLD *in vitro* for our further studies on nutriceuticals.

Conclusion: We provide the first indications for the liver-on-chip model as a physiologically relevant model for complex diseases like NAFLD and how to study such a disease in our platform. Future perspectives include adding liver resident macrophages ("Kupffer cells") to recapitulate the inflammatory cross-talk present in NAFLD. Ultimately, we aim to use this model to assess NAFLD therapeutics as well as assess the impact of patient specific mutations on NAFLD.

Presentation: Poster

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Capturing biological complexity in a colorectal cancer-on-a-chip model

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The biochemical and biomechanical complexities of the tumor microenvironmental milieu are often understudied due to a lack of relevant preclinical model systems. Here we describe a patient-derived microfluidic organ-on-chip platform that incorporates tissue-tissue interfaces and physical forces to aid in the examination of colorectal cancer (CRC) progression. Specifically, we created tumor organoids-on-chips from 5 CRC patient tumors varying in stage and molecular alterations. Tumor organoids were fragmented and seeded in the top channel to form an epithelial layer, while human intestinal microvascular endothelial cells (HIMECs) were seeded in the bottom channel to form a tube-like structure mimicking the vasculature, which resulted in an epithelial:endothelial tissue:tissue interface separated by a porous membrane. Autologous cancer-associated fibroblasts (CAFs) were layered in between extracellular matrices in the top channel to provide tumor-stromal cell interactions. Inter-patient heterogeneity was detected by morphology differences of the epithelial layer using live-cell imaging and metabolite levels in the effluent measured by mass spectrometry-based metabolomics.

A major advantage of this model is the ability to mimic physical forces found in the intestine, including shear stress from fluid flow and cyclic strain, to simulate peristalsis. These physical forces induce changes to the CRC milieu in the organ-on-chip model, including an induction in neurotransmitter levels and epithelial-mesenchymal transition (EMT) when peristaltic-like motions are present. Particularly, cyclic stretching led to significant increases in the secretion of gamma-aminobutyric acid (GABA) by CRC cells. Interestingly, on-chip tumor cell invasion, where cells traverse from the epithelial channel through the porous membrane and into the vascular channel, was also increased in this condition. Inhibitors targeting the GABA-A receptor reversed the observed tumor cell invasion phenotype in the stretched condition. Moreover, GABA agonists promoted invasion in the not-stretched condition, implicating peristalsis-mediated tumor cell invasion with neurotransmitter release. Further studies suggest pharmacological and genetic knockdown of 4-aminobutyrate aminotransferase (ABAT), an enzyme responsible for catabolism of GABA, can reduce the invasiveness of CRC cells, exposing the significance of GABAergic signaling in the CRC environment. This work highlights the importance of recapitulating the organ-level milieu in preclinical studies to reveal novel findings by studying cancer in context.

Presentation: Oral

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Design, development and validation of TToP – True Tissue on Platform: A modular, versatile microphysiological platform for compartmentalized cultures of tissue barriers

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Microphysiological systems apply fluid flow to provide nutrients and stimulations to biological samples, emulating the dynamic *in vivo* microenvironment. However, their traditional "closed-well" design limits the accessibility to the biological sample, resulting in a complex integration in standard laboratory procedures [1]. To overcome these limitations, a platform called True Tissue on Platform (TToP) has been developed. TToP is a modular and versatile platform based on a thin cartridge, which hosts the biological sample, and different modules designed to apply controlled culture conditions. Firstly, the cartridge can be placed in an "open-well" static module to prepare the *in vitro* model with standard procedures. Then, it can be plugged in a "closed-well" perfusion module, to apply recirculating/dynamic conditions, ultimately providing a more physiologically-relevant tool for barrier tissues *in vitro* modeling. To investigate platform functions, Caco-2 intestinal epithelial cells were cultured in the "open-well" static module using DMEM with 10% FBS, 1% Pen-Strep, 5% gentamycin, and non-essential amino acids. After two weeks, the static module was plugged in the "closed-well" perfusion module, enabling apical and basolateral recirculation of culture media, generated by piezoelectrical micropumps. The procedure for shifting from static to dynamic conditions was optimized, checking cell presence and procedural time. After 24 hours, cells were stained with Acridine Orange and Propidium Iodide, labeling living and dead cells, respectively. Less than 10% of dead cells was found. Then, Caco-2 cells were cultured for 3 days in dynamic conditions and time-lapse videos were recorded using a digital microscope. Static controls were run in parallel. At the end of the culture, the cartridges were retrieved, junctional and surface markers (ZO-1, Villin) were visualized by fluorescence microscopy and cell density evaluated by DAPI-labeled nuclei count, comparing static and dynamic conditions. In conclusion, the platform modularity allowed to perform sequential treatments on the same sample and the controlled cartridge retrieval enabled confocal microscopy analysis (30X) maintaining the tissue morphological structure integrity. The combination between TToP modularity and standardization will enable to

recreate the dynamic barrier tissues microenvironment, with minimal handling requirements, paving the way for a faster MPS integration in laboratory procedures.

Reference

[1] Ramadan, Q. et al. (2020). doi:10.1063/5.0011583

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Establishment of a fully human iPSC-derived model of peripheral myelination

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Myelination and node of Ranvier formation play an important role in the rapid conduction of nerve impulses, referred to as saltatory conduction, along axons in the peripheral nervous system (PNS). We report a fully human model of peripheral myelination using human induced pluripotent stem cell (iPSC)-derived Schwann cells (SCs) and motoneurons in a fully defined serum-free medium, to allow disease modeling, drug discovery, and potentially a platform for personalized medicine. Flow cytometry indicated that 88.31% of iPSC-derived SCs expressed a key transcription factor for myelination, early growth response protein 2 (Egr2). After 30 days in coculture, immunocytochemistry was used to visualize key features of myelination, myelin segment and node of Ranvier formation. Myelin basic protein was observed surrounding neurofilament-stained motoneuron axons, clusters of voltage-gated sodium channels and the paranodal protein contactin-associated protein 1 were present, indicating node of Ranvier formation. High resolution confocal microscopy enabled 3D reconstructions of multiple myelin segments for the measurement of myelin g-ratio, a measurement typically collected using transmission electron microscopy, a technique which is difficult to apply to 2D cellular models. The average g-ratio of myelin segments in the wildtype iPSC-derived model of myelination was 0.65, which is within the value range reported in literature from *in vivo* observations. Additionally, the myelination co-culture has been integrated onto a microelectrode array platform to enable functional data collection of conduction velocity along axons. Establishment of this iPSC-based disease model provides a platform to test various drugs and therapeutics that could potentially ameliorate deficits in diseases such as Charcot-Marie Tooth disorder, Guillain-Barre syndrome, and anti-myelin-associated glycoprotein peripheral neuropathy, with the potential for direct translatability to human patients. The integration of this model into other microphysiological systems would provide a functional readout in addition to the collected biomarker information which would be extremely useful for the testing of new therapeutics to treat diseases where the myelination, and therefore action potential conduction, is impaired.

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A proposal for validation of microphysiological systems

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Recently a great number of possibilities for the use of micro-physiological system (MPS) in different ways, such as basic research, kinetics, individual medicine etc., including as replacement of animal use in systemic toxicity, efficacy assessment of chemicals, ADME study, being applied to research, development and control of Chemicals has been proposed. These different applications include the use of primary cells or IPSC-derived cells. These uses go from a simple cell culture up to a transepithelial resistance measurement always trying to predict safe and efficient evaluation of human exposure to chemicals. For a scientific use it is necessary that the model should be validated in order to provide safe and reliable results. One of the possibilities is to compare the results with animal research, but comparison with human data should be more useful. Considering the complexity of MPS, since it is possible to use healthy cells, tumour cells, different flow directions etc., it seems to be very difficult to follow the OECD Guideline 34. So, we propose the validation of MPS to be done by validating the process what means that it will be needed a minimum of process control such as: material of the chip, quality of the cell line, direction, and intensity of the flow etc. The validation of a MPS tool alone seems not to be enough to guarantee the safety of responses, so, if the process at all is validated, the integrity of the response may be indirectly safe to represent a human response.

References

[1] Ingber, D. E. (2020). Is it time for reviewer 3 to request human organ chip experiments instead of animal validation studies? *Adv Sci (Weinh) 7*, 2002030. doi:10.1002/advs.202002030

[2] Rebelo, S. P., Dehne, E. M., Brito, C. et al. (2016). Validation of bioreactor and human-on-a-chip devices for chemical safety assessment. *Adv Exp Med Biol 856*, 299-316. doi:10.1007/978-3-319-33826-2_12

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An MPS CAR-T cell therapy model of the immunosuppressive solid tumor microenvironment

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Chimeric Antigen Receptor (CAR)-T cell therapy has demonstrated remarkable success in treating B cell-based tumors; however, success has not translated to solid tumors with immunosuppressive tumor microenvironments (iTME). Delivering CAR-T cell effector function to a solid tumor involves transport in the circulation, recognition of the tumor vasculature, attachment, and extravasation into the iTME, migration and recognition of tumor cells, and tumor cell killing. Each of these steps is targeted by immunosuppressive mechanisms (e.g., tumor-associated macrophages) in the tumor microenvironment, and preclinical mouse models provide limited opportunities to probe these mechanisms to develop counterstrategies. We have created a microphysiological system (MPS) of the iTME that includes all steps of CAR-T cell trafficking and effector function, including immunosuppression. The microfluidic design is comprised of five parallel microfluidic channels. The central channel was coated with a monolayer of endothelial cells to mimic the vasculature. Adjacent to the central channel are wide chambers that were loaded with the DU145 human prostate cancer cell line (DU145-WT – wild type or DU145-PSCA – a clone that overexpresses prostate stem cell antigen (PSCA)) with or without primary peripheral blood monocytes polarized to an immunosuppressive M2-like phenotype. Finally, the narrow outer channels collect the interstitial fluid flow from the tissue. CAR-T cells specific for the PSCA antigen (or non-specific control) were then introduced in the vascular conduit with or without anti-PD-L1 antibody, Atezolizumab. Over 3 days, tumor growth increased by 1.5-fold, which increased to 2.5-fold in the presence of macrophages. In the absence of macrophages, CAR-T cells actively attached to the endothelium, extravasated, and stopped tumor growth. In the presence of macrophages, CAR-T cell trafficking was significantly reduced from 243 ± 22 CAR-T cells/chamber to 18 ± 8, and cell death (visualization of DRAQ7) was reduced from 666 ± 5 cells to 235 ± 17 cells. In the presence of Atezolizumab, CAR-T trafficking recovered to 70 ± 15 cells/chamber and cell death to 500 ± 37 cells. We conclude that M2-like macrophages limit CAR-T cell trafficking and effector function, and this effect is partially PD-L1-dependent, supporting the premise of combination therapy (CAR-T cells and immune checkpoint blockade) for the treatment of solid tumors.

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Understanding dynamic immune responses within a 3D microfluidic model of human skin

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Dynamic communication between tissue resident cells and circulating immune cells, such as monocytes, orchestrates the skin's responses to infection and plays an important role in tissue repair and regeneration. However, the factors that drive monocyte recruitment into human skin during inflammatory events and the specific signals that direct monocyte fate are poorly understood. Current 3D *in vitro* models recapitulate the basic structure of skin by fabricating dermal and epidermal like layers but do not effectively model the complex and dynamic interactions between the tissue and the immune system. Therefore, the aim of this research is to gain new mechanistic insights into inflammatory responses within human skin, through the development of a novel immune-responsive *in vitro* model.

A 3D printed gelatin template was first used as a sacrificial material to fabricate a microfluidic channel (500 µm diameter) within a fibrin hydrogel embedded with human dermal fibroblasts. NTERT keratinocytes were seeded on top to create an epidermal layer and human umbilical vein endothelial cells were used to line the microchannel and mimic the vasculature. Activation of inflammasome signalling in the epidermal layer with lipopolysaccharide and nigericin stimulated secretion of IL-1 and IL-18 by the keratinocytes, and when CD14+ primary monocytes were then injected into the microchannel, inflammasome activated samples promoted rapid migration of monocytes across the endothelium and into the engineered skin model. Further analysis revealed that monocyte recruitment was specifically dependent on signalling from the keratinocytes but also restrained by the endothelial barrier.

These findings demonstrate that the developed microfluidic skin model has immune responsive capabilities and could be used to investigate human specific inflammatory responses within the skin. On-going studies aim to further assess monocyte fate and differentiation via flow cytometry and single cell transcriptomics as well as their influence on skin homeostasis and remodelling.

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CBD prevents TNF-induced barrier disturbance in intestinal epithelial cells

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Outline: The non-transformed cell line IPEC-J2 closely represents porcine jejunal epithelium and has been characterized as a suitable *in vitro* model for analyzing physiological changes in epithelial barrier function [1]. Current studies focus on the beneficial effects of secondary plant compounds on epithelial barrier function to treat or prevent intestinal inflammatory diseases. One promising candidate is cannabidiol (CBD), which is extracted from *Cannabis sativa* and is currently discussed to have anti-inflammatory effects [2]. However, data regarding the effects of CBD on porcine intestinal barrier disturbance due to inflammation is still lacking. Therefore, we aimed to analyze if CBD might attenuate or prevent tumor necrosis factor (TNF)-induced disruption of porcine epithelial barrier function using IPEC-J2 cells.

Methods: IPEC-J2 cells were cultured onto semipermeable filter supports and incubated with TNF (1000 U/mL) in the presence or absence of CBD (40 mM). Barrier integrity was analyzed by transepithelial resistance (TER) and mannitol flux, representing paracellular permeability. Subsequently, cells were further processed for molecular analysis of specific receptors.

Results: Incubation of IPEC-J2 cells with TNF for 48 h significantly decreased TER (ctrl: 89.89 ± 2.01%, TNF: 69.64 ± 2.76%, ***$p < 0.001$, $n = 16$) and enhanced paracellular permeability (ctrl: 0.17 ± 0.01 nmol/cm^2/h, TNF: 0.29 ± 0.02 nmol/cm^2/h, ***$p < 0.001$, $n = 5$). The addition of CBD prevented the TNF-induced changes (TER: 84.72 ± 4.58%, *$p = 0.03$, $n = 16$; paracellular permeability: 0.24 ± 0.01 nmol/cm^2/h, *$p = 0.02$, $n = 6$). A tendentiously enhanced expression of the specific receptor TNFR-1 by TNF could also be mitigated by co-incubation with CBD (ctrl: 100%, TNF: 126.19 ± 26.15%, TNF + CBD: 104.06 ± 23.62%, $n = 8$), without being significantly different. Confocal laser scanning immunofluorescence microscopy revealed an altered expression and localization of specific receptors in the presence of CBD.

Conclusion: CBD prevented the barrier-disturbing effects of TNF in IPEC-J2 cells. This gives rise to further experiments, analyzing the barrier-strengthening effects of CBD on a functional and molecular level in more detail.

This study was supported by a grant of the German Research Foundation, AM141/11-2.

References

[1] Droessler, L., Cornelius, V., Markov, A. G. et al. (2021). *Int J Mol Sci 22*, 8746.

[2] Hasenoehrl, C., Storr, M. and Schicho, R. (2017). *Expert Rev Gastroenterol Hepatol 11*, 329-337.

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Bone marrow-on-a-chip: Emulation of the human endosteal and vascular hematopoietic stem cell niche

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In adults, the bone marrow is the location of the hematopoietic system. The hematopoietic stem cell niche (HSC) constitutes an integral component and enables the lifelong maintenance of HSC as a source for producing new blood and immune cells. The niche is divided into endosteal and vascular compartments with specific functions in the maintenance of the HSCs. The endosteal and vascular niche support the proliferation, homing, self-renewal, and differentiation with contradicting studies regarding their primary individual functions.

Recently we developed a bone human marrow on-a-chip system suitable for the long-term culture of HSC preserving the native stem cell phenotype. The organ chip is based on a ceramic scaffold seeded with mesenchymal stromal cells that, upon generation of endosteal niche-simulating microenvironments, ensure the HSC culture for up to 8 weeks. To further increase the complexity, we integrated endothelial cells to emulate the vascular niche in addition to the endosteal niche environment. The comparative analysis demonstrates that the endothelialized model maintains HSCs and supports the proliferation of HSPC cells likewise but induces a more pronounced differentiation of the stem cells into the myeloid lineage.

The endothelialized human bone marrow on-a-chip model can reduce animal experiments regarding HSC niche biology and drug testing for cancer research and provides the foundation for developing derived disease models.

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3D-oxygen gradient chip for cancer cell migration research

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Most breast cancer related deaths are not caused directly by the primary tumor, but by secondary tumors formed through metastasis to other organs [1]. In the first step of metastasis, invasion, cancer cells migrate from the primary tumor into the surrounding stroma. This process is determined to a large extent by properties of the tumor microenvironment [2], and hypoxia is reported to be an important factor [3]. Here, we introduce a 3D-oxygen gradient chip to study the effect of hypoxia on the migration of cancer cells in 3D. The chip is made of PDMS, which is permeable to oxygen. A multi-staged cell culture chamber is molded on one side of the chip, while an oxygen leaching channel is molded on the opposite side. Both sides are covered by a glass slide and a PMMA plate which are impermeable to oxygen. The oxygen gradient is realized by the continuous scavenging of oxygen by the flow of a sodium sulfite (Na_2SO_3) solution in the leaching channel underneath the cell culture chamber, while oxygen keeps diffusing into the other side of the chip from the atmosphere. After 24 hours of the continuous sodium sulfite solution flow in the leaching channel, the oxygen distribution within the chamber reaches a steady state. At that point, a stable oxygen concentration gradient is present that linearly increases from 1% to 11% from the leaching channel side to the open side of the chip. Human breast cancer cells (MDA-MB-231) suspended in type I Collagen hydrogel can be seeded into the cell culture chamber to observe their response to different oxygen conditions within the chamber. Currently, we have found that the viability of cancer cells increases from the hypoxic region to the physioxic region. The next step is to study the morphology, protein secretion, HIF-1α, and motility of the cancer cells in different regions of the cell culture chamber.

References

[1] Chaffer, C. L. and Weinberg, R. A. A. (2011). *Science 331*, 1559-1564.
[2] Friedl, P. et al. (2011). *Cell 147*.
[3] Palacio-Castañeda, V. et al. (2022). *Lab Chip 22*.

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3D culture of blastocyst like structures derived from pluripotent stem cell cultures

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Understanding the molecular mechanisms that underline blastocyst formation and implantation is critical for improving the efficiency of assisted reproductive technologies. Recent advancements *in vitro* embryo models derived from human and mouse stem cell cultures have opened new avenues for understanding the mechanistic insights into early lineage segregation and implantation. Here we have developed two strategies for the generation of blastocyst-like structures (blastoids) from two ungulate species, Bos taurus and Ovis Aries: 1) 3D differentiation and self-organization (SO) directly from naïve-like bovine embryonic stem cells (bESCs), and 2) 3D assembly (AS) of bovine trophoblast stem cells (bTSCs) and naïve-like bovine ESCs. These blastoids can be generated with high efficiency and resemble bovine blastocysts in terms of morphology, size, cell number, lineage composition, and allocation, as revealed by immunostaining of epiblast (SOX2), hypoblast (SOX17,), and trophectoderm (CDX2) markers. To evaluate their developmental competence, we evaluated the *in vitro* growth of the blastoids and blastocysts under a 3D rotating culture rotating culture system (Clinostar Incubator, Celvivo). We found trophoblast cells and cavities in both IVF blastocysts and blastoids continued to proliferate and expand over a period of more than 2 weeks, which were also accompanied by an increase in the ICM size and a maturation of trophectoderm markers. This 3D *in vitro* culture of blastocyst and blastocyst like structures creates a unique opportunity to study the early stages of embryonic development.

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Emerging networks in Berlin: Charité 3R, *Der Simulierte Mensch* and Einstein Center 3R

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With its large number of research institutes, Berlin is a center of biomedical research with an ever-growing and vibrant MPS community.

Since 2018, three new beacons of 3R research have emerged in Berlin on the initiative of networked scientists and with financial and political support, realizing that modern alternative methods have enormous potential for advancing basic biomedical research and translation.

Charité established Charité 3R to anchor 3Rs in the internal biomedical science structurally. Together with *Technische Universität Berlin*, both institutions acquired funding for a new research building, "*Der Simulierte Mensch*," opening in 2024, focusing on alternative methods to animal experiments. Furthermore, in 2021, universities and non-university partners established the Berlin-wide Einstein Center 3R (EC3R).

Charité 3R is active in three pillars: Scientific and political communication on animal experiments and alternatives; education and support to promote the 3Rs; direct research funding in all 3R aspects. Since 2018, almost 6 Mio € have been provided for 3R research projects.

Der Simulierte Mensch will be a joint research center of TU Berlin and Charité. It houses interdisciplinary scientific projects of excellent national and international researchers and engineers aiming to simulate human physiology at subcellular, cellular, and organ/organoid levels. A central feature is two publicly open floors for science communication and outreach to and from the scientific community.

The Einstein Center 3R (EC3R) aims to strengthen Berlin's 3R activities by networking. It includes measures in communication, training, and research. The latter focuses entirely on developing robust 3D cell culture models as an alternative to animal testing.

All these initiatives work highly intertwined with developing high-quality and forward-looking NAMs (new approach methodologies) for biomedicine. In addition to ethical and legal reasons, there are strictly scientific reasons for consistently pushing forward alternatives. The presented 3R community can significantly strengthen Berlin as a science location by opening scientific perspectives, promoting spin-offs, and seeking international cooperation. We offer networking opportunities to international researchers interested in collaborations with Berlin's MPS and 3R community and support local scientists to get involved in the MPS research environment.

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Raman-on-chip: A window for the marker-free observation of tumor-immune interactions

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The tumor-immune interaction plays a crucial role in tumor prognosis and therapy resistance. However, the biological processes involved in tumor-immune interaction are highly complex. To enable efficient testing with a great expressive power, model systems, mimicking the therapeutic response as close to physiological reality as possible are of utmost importance. The combination of Organ-on-Chip (OoC) platforms and non-invasive imaging techniques offer great promise to serve as window for marker-free real-time monitoring and investigations of dynamic cellular processes at spatial and temporal resolution.

Raman microspectroscopy and imaging (RMS) can access various cell and tissue structures due to their unique molecular-sensitive spectral fingerprints. Our aim is to enable the real-time observation of tumor-immune interaction and the tumor responsiveness to different cancer therapies in-chip. Therefore, a microscopically accessible OoC platform was designed, and RMS will be implemented on single cells, tissue sections and living 3D tissues to differentiate circulating and tissue-resident immune cell subsets.

As a first step, reference spectra of different immune cell subtypes were generated via immunofluorescence-guided RMS on tissue sections of patient-derived microtumors (PDMs). Current focus resides on the identification of monocyte derived cells and lymphocytes, where it was previously shown that Raman signatures are sensitive to discriminate between pro- and anti-inflammatory macrophages. Further immune subtypes are being investigated.

In the future, the protocols will be translated into 3D tissue samples in microphysiological OoC models. These readouts will enable the *in situ* monitoring of tissue-infiltrating immune cells in

a patient-derived physiologically relevant *in vitro* model and unravel information about the principles and dynamics of tumor-immune interaction.

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Modeling ischemic stroke in a tri-culture neurovascular unit on-a-chip

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In ischemic stroke, the function of the cerebral vasculature is impaired. This vascular structure is formed by the so-called neurovascular unit (NVU). The NVU is crucial in maintaining brain homeostasis and healthy functioning of the central nervous system (CNS). A better understanding of the mechanisms involved in NVU dysfunction and recovery may lead to new insights for the development of highly sought therapeutic approaches. To date, there remains an unmet need for complex human *in vitro* models of the NVU to study ischemic events seen in the human brain.

We here describe a human NVU on-a-chip model using a platform that allows culture of 40 chips in parallel. The model comprises a perfused vessel of primary human brain endothelial cells in co-culture with induced pluripotent stem cell derived astrocytes and neurons. The model displays expression of endothelial adherens- and tight junction proteins as well as astrocytic and neuronal markers. In addition, the model presents with relevant brain endothelial transporters and shows spontaneous neuronal firing. Tight barrier function was evidenced by retention of the small molecule sodium fluorescein in its lumen and allows study of compound-induced barrier disruption.

Ischemic stroke was mimicked in the NVU model using a threefold approach that combines chemical hypoxia, hypoglycemia, and halted perfusion. The resulting cultures showed reduced BBB integrity, lowered mitochondrial membrane potential, and reduced adenosine triphosphate levels, which are common features of ischemic stroke.

The NVU on-a-chip model presented here can be used for fundamental studies of NVU function in stroke and other neurological diseases and for investigation of potential restorative therapies to fight neurological disorders. Due to the platform's relatively high throughput and compatibility with automation, the model holds potential for drug compound screening.

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Automation and validation of the OrganoPlate LiverTox for hepatotoxicity detection

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Drug-induced liver injury (DILI) is one of the leading causes of market withdrawal in the pharmaceutical industry and poses a serious health risk to affected patients. Identification of hepatotoxic compounds in the preclinical phase of drug development is key to preventing DILI, however currently employed animal and two-dimensional (2D) *in vitro* models do not adequately predict human hepatotoxicity. Existing models suffer from many challenges including species differences, throughput limitations, and lot-to-lot variability of primary human hepatocytes. Here, we developed a functional 3D *in vitro* model of the human liver, the OrganoPlate LiverTox compatible with automated liquid handling and validated for hepatotoxicity screening. To build the model, up to 96 independent 3D perfused cultures were established on MIMETAS' OrganoPlate 2-lane using automated liquid handling to seed cells, dose, collect media and add assay reagents. For cell seeding, induced pluripotent stem cell-derived hepatocytes (iHep) in extracellular matrix were added to a microfluidic channel, following which endothelial and Kupffer cells were added to an adjacent channel to mimic the liver sinusoid. Characterization of the model revealed long-term hepatocyte function including CYP3A4 activity, as well as albumin and urea production for up to 14 days of culture. Fetal hepatocyte marker alpha-fetoprotein (AFP) dramatically declined over the 14 day culture, supporting iHep maturation in the OrganoPlate LiverTox. Assay validation studies using troglitazone as a positive hepatotoxic control compound revealed robust Z-factors ≥ 0.2 for albumin, urea, iHep viability (propidium iodide staining), and iHep nuclear size (Hoechst 33342 staining) assay readouts. Using these assays, 159 compounds of known hepatotoxicity were screened in the OrganoPlate LiverTox (50 µM, 72 h) and ranked by a composite score by combining the assay readouts. A follow-up dose response evaluation of select hits suggested the albumin assay to be the most sensitive readout in calculating TC50 values. Taken together, the OrganoPlate LiverTox is a promising platform for hepatotoxicity detection and has the potential to be used in a high throughput screening capacity.

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Bioprinting of perfusable organ models for disease modelling

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Bioprinting allows the generation of 3D organ models with high spatial resolution. We have produced models for infectious diseases [1] and cancer [2] to study disease mechanisms and to test drug candidates. To improve the physiological relevance of the models, vascular structures have been included in the models. The tubes were connected to a peristaltic pump or a micropump to perfuse models with a media stream and thereby guarantee supply of nutrients and oxygen. Physiological characterization clearly demonstrates higher viability of cells in perfused models compared to static cultivation. Bioprinting technologies allow inclusion of human tumors into a microenvironment composed of human cells. These systems are superior to mouse models into which human tumors are implanted and which then represent chimeric artifacts. We have demonstrated that the bioprinted tumor models can be used to distinguish cytostatic agents with high specificity for the tumor cells and those with a general toxicity, which is also harmful to healthy tissue surrounding the tumor. The inclusion of a vascular system permits longer cultivation of the models compared to static systems, and it also simulates physiologically relevant application of the substance to be tested in the disease model. We demonstrate that perfused, bioprinted models cannot only be used to determine efficiency of cytotoxic substances, but also to study their mode of action such as induction of apoptosis. In the next step, vascularized cancer models will be connected to other organ systems.

References

[1] Berg, J., Weber, Z., Fechler-Bitteti, M. et al. (2021). Bioprinted multi-cell type lung model for the study of viral inhibitors. *Viruses 13*, 2030.

[2] Wu, D., Berg, J., Arlt, B. et al. (2021). Bioprinted cancer model of neuroblastoma in a renal microenvironment as an efficiently applicable drug testing platform. *Int J Mol Sci 23*, 122.

[3] Mei, Y., Wu, D., Berg, J. et al. (in preparation). Generation of a perfusable 3D lung cancer model by digital light processing.

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ALS-on-a-chip: Towards patient-derived models for personalized therapy development

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons with an estimated lifetime risk of 1:400. ALS disease mechanisms are poorly understood, but motor axon aberrances are increasingly gaining interest as therapeutic targets. Here we describe a 3D motor neurite outgrowth model that can be leveraged to study axonal biology. Human iPSC-derived motor neurons were grown embedded in extracellular matrix in the OrganoPlate®, a 3D microfluidic culture platform that allows culture of 40 chips in parallel. The neurons extend their axons into an adjacent layer of gel, while the dendrites and soma remain in the somal compartment. The model was tested for its ability to recapitulate nerve damage caused by vincristine. Toxicity was assessed after 96 hours of exposure, for a concentration range of 0.001-0.01-0.1-1-10-100 nM. The toxic effects were assessed by labeling the neuronal networks with green-fluorescent calcein-AM and imaging using a high content confocal microscope. The outgrowth in the axonal compartment was quantified as a measure of toxicity. High concentrations 1-10-100 nM resulted in a more than 50% disruption of the axonal network. Lower concentrations did not induce damage. We then assessed the axonal network following a 7-day exposure to 5 mM of glutamic acid, mimicking excitotoxic conditions that influence ALS pathogenesis. Outgrowth was significantly reduced following glutamic acid exposure, while the number of stress granules was upregulated. These results demonstrate that the model is sensitive to compound-induced axonal toxicity, while the results from the vincristine exposure demonstrate that the model can be used for the generation of dose-response curves. In conclusion, our platform can be used to study toxic effects on peripheral neurons, and for the study of ALS-relevant processes. The 384-well format of the OrganoPlate® renders the model easily scalable and automation-compatible. The model will therefore be useful for studying axonal biology and disease and subsequent therapy development through drug screenings.

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Multi-hydrogel microvasculature by 2-photon polymerization and scaffold micromolding on-chip for perfusable cell co-culture

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Introduction: Microphysiological systems offer human *in-vitro* models as an alternative to animals in biomedical research [1]. Despite the hydrogel integration in such systems has paved the way for 3D cell culture, engineering vasculature networks in hydrogels still represents a challenge, in terms of material processing and device interfacing [2]. In this work, we propose a new strategy to generate a multi-hydrogel vascular network at a relevant tissue length scale integrated into a microfluidic device to allow perfusion and cell monitoring.

Materials and methods: The developed strategy consists of a two-step process. Firstly, a hydrogel vasculature-like network with a 40 µm-thick channel wall is printed inside a microfluidic device with a gelatin-based ink by 2-photon polymerization. Then, a second cell-laden hydrogel, here fibroblasts in fibrin, is injected around the printed structure. The vascularized system is finally obtained by seeding HUVECs into the printed hydrogel channels. The on-chip integration of all the fabrication steps removes potential structure damage while ensuring a reliable connection with an external pump for perfusion.

Results and conclusion: The proposed strategy allowed a multi-hydrogel vascular network inside a microfluidic platform with a capillary-like feature size (10 µm diameter). The hydrogel integration inside the microfluidic chip ensured the perfusion to sustain HUVEC and fibroblast co-culture for up to 10 days with HUVEC monolayer formation within 48 hours. Combining a printed thin-wall hydrogel channel network and hydrogel micromolding drastically reduced the printing time (10 min), the structure deformation due to hydrogel swelling and cytotoxicity as no cells were involved during the fabrication process. Moreover, the 2-step approach allowed the engineering of a vasculature network inside natural hydrogels commonly not suitable for high-resolution bioprinting. The implementation of the presented strategy on a microfluidic device offers the capability to generate perfusable multi-hydrogel microphysiological systems for new 3D co-culture *in-vitro* models.

This research was funded by ERC(757444) and KAW (WAF 2016.0112).

References

[1] Huh, D., Hamilton, G. A. and Ingber, D. E. (2011). From 3D cell culture to organs-on-chips. *Trends Cell Biol 21*, 745-754.

[2] Zhang, Q., Bosch-Rué, È., Pérez, R. A. et al. (2021). Biofabrication of tissue engineering vascular systems. *APL Bioeng 5*, 021507.

Presentation: Poster

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3D microphysiological placenta *in-vitro* model as a tool for drug transport studies and risk assessment

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The human placenta is a unique organ that acts as the interface between the mother and the fetus. Its multilayered structure that consists of the syncytium and the fetal endothelium, separated by a thin connective tissue, is essential for the exchange of various substances and to protect the fetus from xenobiotics. Considering that over the past decades the intakes of medication during pregnancy are on rise, risk assessment of drug therapies during pregnancy is gaining more importance. Unfortunately, the current widely used *in vitro* translocation models do not fully resemble the physiological structure and fail clinical translation. Here we present a *in vitro* model of the placental barrier toward the assessment of drug transport using the microfluidics OrganoPlate® 3-lane. This platform is based on a 384-microtiter plate which allows for a parallel culture of 40 perfused miniaturized placenta barrier, in a membrane-free manner.

To model the placenta barrier on-a-chip, the trophoblast cell line BeWo b30 and primary human vascular endothelial cells HUVEC were grown against tissue-like extracellular matrix (ECM) in the OrganoPlate®. Perfusion through the lumen of maternal epithelium and fetal endothelium is induced without pump and can be controlled to model mechanical cues. Syncytium formation, polarization and specific marker expression were characterized at the protein and mRNA level. Upon perfusion flow, trophoblast and endothelial cells formed lumenized, leak-tight confluent tubular structures. Barrier tissue integrity was confirmed through TEER (transepithelial electrical resistance) measurements. Syncytium formation was characterized by a breakdown of tight junction (ZO-1), and an increase in hCG-β expression. The activity of major placenta barrier drug transporters was evaluated through in- and efflux transporter assays. Finally, drug-induced toxicity was assessed by syncytium apical exposure to common drugs taken during pregnancy, known substrates

of MRP. Cellular damage was assessed, and barrier disruption was evaluated by TEER measurements using the OrganoTEER®. Exposure of the maternal compartment to compounds showed dose-dependent cellular damage and barrier integrity loss.

The placental barrier on-a-chip provides a complex co-culture model to recreate an *in vivo*-like microenvironment. Suitable for transport studies and high-throughput drug-induced toxicity screening, this model provides valuable insight for drug risk assessment during pregnancy.

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Understanding hemolysis-induced lung injury using an advanced preclinical *in vitro* model

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Sepsis and sepsis-induced acute respiratory distress syndrome (ARDS) are often associated with hemolysis and, consequently, with increased levels of cell-free hemoglobin (CFH) in the bloodstream CFH and its highly oxidative degradation product, heme, become toxic and contribute to further impaired lung function and gas exchange at the alveolar air-blood barrier.

In 2016, a study from 50 countries indicated that 10% of ICU patients and 23% of mechanically ventilated patients fulfilled the criteria for ARDS, representing a major clinical problem worldwide currently accentuated by COVID-19. Due to its complex etiology and high patient heterogeneity, advanced and realistic preclinical experimental models are needed to understand better the pathomechanisms associated with ARDS.

In this study, we developed an advanced alveolar barrier model replicating relevant hallmarks of the effects of hemolysis in the distal lung. As previously described, the immortalized alveolar epithelial (AXiAECs) and lung microvascular endothelial cells were co-cultured on the AXLung-on-chip system. Cells were treated with either hemoglobin or heme from the vascular compartment to resemble hemolytic events, and effects on barrier permeation (Papp FITC), function (trans-barrier electrical resistance, TER), inflammation, and detoxification (secreted protein screening, qPCR) were investigated.

While hemoglobin led to mild effects on barrier function on-chip (~20% TER reduction) and incipient proinflammatory effects (increased GROα, IL-6, IL-8), treatment with heme resulted in a more pronounced barrier disruption (~80% TER reduction), increased permeability, altered secretion of proinflammatory mediators (IL-8, MCP1, ICAM1, IL-1a, VCAM1), impaired secretion of factors involved the coagulation cascade (tPA, PAI-1) and activation of genes associated with heme detoxification and inflammation (HO1, NQO1, CYP1B1). Those effects were accentuated in breathing conditions, underlining the relevance of reproducing mechanical cues, especially when patients often require mechanical ventilation.

Taken together, our findings indicate that hemoglobin breakdown and the resulting release of heme is crucial in hemolysis-associated lung injury. This advanced *in vitro* model mimics key features of hemolysis-induced lung injury.

Presentation: Poster

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Establishment of an *in vitro* 3D model of microvascular perfused cardiomyocytes

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More than 64 million people worldwide suffers from heart failure [1]. Patients presenting with heart failure with preserved ejection fraction constitute a large unmet need given the paucity of treatment options and rapid increase in incidence and prevalence. To develop new treatments model systems with higher translational value are needed, since traditional 2D mono-culture systems fail to recapitulate important physiological features of the human heart. To be able to study the complex interactions in the heart and to test new drug candidates related to cardiac pathology more advanced models, that incorporate all the relevant cardiac cell types in a 3D environment with perfusion through microvascular networks are needed. We have established a 3D co-culture system that consist of a vascularized network of human primary endothelial cells, fibroblasts and macrophages that recapitulates some of the key physiological features. Fibroblasts are important as they produce extracellular matrix and serve a supportive role in the tissue [2], while macrophages promote homeostasis by apoptotic cell removal and work as facilitators of endothelial bridging [3].

We first established 3D co-cultures in PEG-based hydrogels. Our preliminary results indicate that the crosstalk between fibroblasts and macrophages influence the ability of the endothelial cells to form connective networks. Furthermore, when co-culturing all three cell types in 3D hydrogels, the expression pat-

terns of the extracellular matrix proteins collagen I and fibronectin were different from the cultures without macrophages.

We then incorporated flow to the model inside an organ-on-a-chip system giving us the opportunity to study the effect of different flow rates and exogenous stimuli to mimic both healthy and diseased states. This also enable us to test different drug candidates and determine if they have effects on the perfusion, barrier integrity and 3D organisation.

By establishing a perfused microvascular system, we aim to combine this system with human IPSC-derived cardiomyocytes to assess changes in contractility when challenged with new drug candidates, thereby providing a multicellular 3D platform that recapitulates key physiological features of the human heart.

References

[1] 2022. doi:10.1093/cvr/cvac013
[2] 2021. doi:10.1016/j.cell.2021.06.024
[3] 2021. doi:10.1007%2Fs10741-021-10156-z

Presentation: Poster

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Studying the therapeutic potential of live microbes and antifungals *in vitro*: An intestine-on-chip approach

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The yeast *Candida albicans* is a normal member of the human microbiota, colonizing mucosal surfaces, such as the intestine of most humans. However, the intestine is also a main reservoir of fungi causing systemic candidiasis. Upon certain predisposing conditions, *C. albicans* can become pathogenic and translocate through the epithelial barrier into the bloodstream, causing life-threatening infections.

Several *in vitro* and *ex vivo* infection models have been developed to study the complex interactions of *C. albicans* with the human host, including mucosal surfaces, epithelial barriers, blood, and immune cells. These models are instrumental to understand specific interactions, but they fail to provide a comprehensive overview of the infection process. Therefore, our laboratory has used a previously established intestine-on-chip [1] model in the Dynamic42 biochip to mimic the most common niche of *C. albicans* and to investigate the initial steps during invasion and translocation through the intestinal epithelium leading to systemic candidiasis.

C. albicans is inoculated in the intestinal compartment, which includes a 3D tissue with villus and crypt-like structures. As the infection progresses, the fungus invades through the epithelial barrier, interacts with the resident immune cells in the model, and translocates to the vascular compartment. An image-based bioinformatic pipeline is applied to immunofluorescence images at 12 hours post-infection to obtain quantitative and qualitative data on tissue structure, fungal morphology, and invasion events. Furthermore, by combining the *in silico* analysis with molecular readouts in the model, we can assess the impact of potential prophylactic or therapeutic approaches.

Experiments in this model showed that Lactobacillus species antagonize *C. albicans*, reducing host damage and translocation to the vascular compartment [1]. In addition, a clinically relevant antifungal perfused through the vascular compartment reduced fungal burden and prevented translocation at concentrations equivalent to those found in patients. The same approach allowed us to demonstrate the reduced susceptibility to caspofungin of a resistant clinical isolate of *C. albicans*.

In conclusion, the intestine-on-chip model is a promising tool to dissect *C. albicans* infection patterns and to study the relevance of critical fungal and host factors *in situ* in an *in vivo*-like environment.

Reference

[1] Maurer, M. et al. (2019). *Biomaterials 220*, 119396.

Presentation: Oral

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Automated high-content phenotypic screening and analysis platform to study pre- and post-implantation morphogenesis using stem cell-derived embryo models

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The integration of three-dimensional (3D), bioengineered *in vitro* models with high-throughput generation and high-content imaging and analysis will greatly impact the fields of drug development, embryo-toxicity, developmental biology, and reproductive medicine (Shankar et al., 2021). The use of micro-engineering technologies to guide stem cell self-organization has enabled the generation of *in vitro* models that mimic specific stages of mammalian embryonic development. We recently showed the formation of 3D *in vitro* stem cell-derived embryo models that recapitulate epiblast (Epi) and extraembryonic endoderm (XEn) co-development, later progressing to form a pro-amniotic cavity (PAC), named XEn/EpiCs, mimicking a partial E5.5 embryo (Vrij et al., 2022). This project aims to utilize pre- to post-implantation stage embryo models to allow *in situ* imaging and analysis within polymer film-based microwell platforms. Here, we used thermoformed microwell arrays (Giselbrecht et al., 2006) to perform a phenotypic screen with 40 compounds that activate or inhibit specific signaling pathways involved in the development of XEn/EpiCs. The compounds that showed a significant effect on the occurrence of XEn/EpiCs and displayed modulated phenotypes were shortlisted to study their effect at different time points of exposure. The different phenotypes were classified based on their developmental stage and the proxy sizes of tissue compartments were quantified individually. We show that key pathways during implantation, namely Wnt, Fgf/MAPK, BMP/Tgfβ, and Activin/Nodal, affect the timing and the morphogenetic events leading up to the formation of PAC in our *in vitro* model. We have devised an automated image analysis pipeline using machine learning to quantify the mentioned parameters and compare the morphogenetic effect of different pathway modulators. Our new platform has the potential to become a powerful tool to perform automated large-scale screens to further elucidate the mechanisms governing development. Such a system can be efficiently scaled-up to conduct phenotypic screens of signaling modulators, drugs, or teratogens to generate valuable biological and physiological readouts.

Presentation: Poster

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A novel microfluid liver-on-chip model: Application in regulated genotoxicity testing

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Genotoxicity assessment of test compounds are based on a combination of tests to assess different genotoxic endpoints associated with human diseases: mutagenicity, clastogenicity and aneuploidy. To this day, there is no single genotoxicity test capable of detecting all mechanisms, and all assays involve rodent metabolic activation system or *in vivo* rodents testing, which could lead discrepancy from human accuracy predictivity response.

The objective of this work was to develop a single *in vitro* assay able to accurately address the different *in vitro* and *in vivo* genotoxicity endpoints using a human cells metabolically competent *in vitro* system to increase human-relevant predictivity outcome and replace animals testing. Different models were evaluated, and the most promising evaluated model consists of a fluidic flow microphysiological liver-on-chip system using the PhysioMimix™ barrier plate supplied by CN-Bio co-cultured with human lymphoblastoid TK6 cells in Transwell® insert.

The preliminary results obtained with direct genotoxicants, methanesulfonate and ethyl methanesulfonate, and indirect genotoxicants, benzo[a]pyrene and cyclophosphamide, demonstrated results in line with expected *in vivo* responses for the end points evaluated, namely the comet assay and the micronucleus test. Measured levels of urea and albumin, in addition to the CYP enzymes activity, also demonstrated appropriate liver properties with metabolic competency.

In conclusion, the human cells model showed appropriate metabolic properties competency without requiring additional rodent metabolic activator, and the capability of appropriately addressing genotoxic adverse outcomes within a single system, i.e., induction of chromosomal damage or damage to the mitotic apparatus (micronucleus test) and DNA strand breakage (comet assay).

The next steps in the development of the model will be to integrate the evaluation of mutagenicity by duplex sequencing analysis and to increase the number of compounds evaluated in the model to have a better view of its accuracy.

Presentation: Poster

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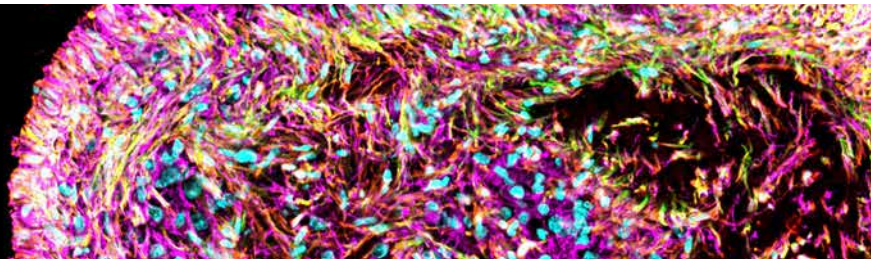

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Development of a 3D bronchial model for application in microphysiological systems containing recirculating neutrophils: *In vitro* assessment of respiratory sensitizer aerosols

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Low-molecular-weight (LMW) respiratory sensitizers are chemicals that cause allergic asthma when repeatedly inhaled, which happens mainly in the occupational environment. Although the EU REACH considers LMW chemical allergens to be Substances of Very High Concern (SVHC), currently there are no validated methods for toxicity/risk assessment of such toxicants. That can be partially explained because the Adverse Outcome Pathway (AOP) has not yet been fully understood, once the *in vivo*-based methods are limited in identifying such compounds, mainly in terms of immune system activation and lung tissue response. In this context, we have previously investigated the effects of seven different LMW respiratory sensitizers upon pulmonary system cells, where we demonstrated that bronchial epithelial, endothelial and monocytic cell lines responded differently to these toxicants regarding activation of inflammatory molecular pathways. Now, this work aims to integrate these different cell lines into a 3D co-culture bronchial epithelial model for further application in a microphysiological system containing recirculating neutrophils, which could be applied for addressing immunological and functional endpoints related to the LMW respiratory allergens mode of action. Transwell inserts for 24-well plates with 8.0 µm pore size were employed for the epithelial model reconstruction. The bronchial epithelial cells BEAS-2B were cultivated in the upper compartment, meanwhile EA.hy926 endothelial cells were grown in the basolateral side of the insert. Results demonstrated that BEAS-2B presented sustained growth and remained viable for 21 days after cultivation in an air-liquid interface. Moreover, EA.hy926 cells have grown in BEAS-2B culture medium for 14 days and presented regular expression of CD31 endothelial biomarker. Besides we also differentiated HL-60 cells into neutrophils using a differentiation protocol based on incubation with dimethylsulfoxide. The results demonstrated that HL-60 cells were successfully differentiated into dHL-60 neutrophils, which has been shown through increased CD18 and CD11b surface biomarkers expression quantification by flow cytometry. In the following steps, we will integrate the epithelial compartment in the microfluidic system containing the recirculating dHL-60 neutrophils and perform the exposure to LMW sensitizers aerosols for further measurement of leucocyte epithelial transmigration, as well as inflammatory biomarkers production and epithelial functional biomarkers modulation.

Presentation: Poster

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Experimental infection of primary hamster airway cells with SARS-CoV-2

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The hamster *in vivo* model of infection has proved a useful tool for studying the pathology of SARS-CoV-2 infection and in the development of new vaccines and therapeutics. SARS-CoV-2 infection in hamsters, as in humans, is predominantly restricted to the airway and lungs. Thus, many early research questions might be answered in *ex vivo* differentiated airway models. In addition, the scientific community has an ongoing need to assess virus variants and *ex vivo* alternatives to animal models such as those described might help reduce overall animal use.

Airway cells, collected from the trachea of Golden Syrian Hamsters, harvested by enzymatic digestion followed by mechanical scraping were seeded directly onto Transwell membranes. The cells were grown as submerged cultures for six days before switching to a differentiation media and exposing the apical surface of the Transwells at the air-liquid interface. The cells were cultured at ALI for a further 21 days before infection with SARS-COV-2 (Victoria isolate).

After infection, Transwell membranes with cells *in situ* were harvested and fixed each day for scanning electronic microscopy (SEM) imaging and immunofluorescent staining for viral nucleoprotein and epithelial cell markers. Cells were also extracted directly from membranes for RNA extraction and cytokine analysis by qRT-PCR. Live viral shedding into the apical and basolateral media were also determined using focus-forming unit assay.

The readouts and images describing the course of SARS-CoV-2 in *ex vivo* differentiated Hamster airway cultures will be presented along with the likely uses of this model.

Presentation: Poster

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Unlocking the secrets of organoids: High content screening device with 3D imaging, machine learning and extreme condition studies

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We have engineered a versatile High Content Screening (HCS) device to streamline all the steps of organoids and organotypic cultures to exploit their full potential in understanding morphogenesis and tissue homeostasis. Our approach is based on a new generation of versatile scaffolding cell culture multiwell chips (JeWells) that enable fast 3D imaging (*Nat Methods*, July 2022). By biofunctionalizing JeWells with extracellular matrix (ECM) components, we can produce precise microniches to grow and differentiate microphysiological elements. The combination of high-resolution 3D microscopy techniques with HCS and machine learning approaches has allowed us to quantitatively describe the morphogenesis of hundreds of living organoids and correlate this with phenotypic characterization to decipher mechanisms involved in human developmental biology and tissue physiopathology. By using large numbers of 3D images to train convolutional neural networks, we can precisely detect and quantify subcellular and multicellular features, such as mitotic and apoptotic events, multicellular patterns, and classify whole organoid morphologies. Our device is fully compatible with classical imaging techniques such as brightfield, widefield, light sheet or confocal microscopy and the defined positioning of organoids allows correlations between all these different techniques without any loss of organoids.

Recently, by utilizing the absence of material loss and the relative confinement provided by our devices, we have been able to study the effect of extreme mechanical conditions on human organoids. We have successfully made a proof of concept that the new version of JeWells, called Zero-G Wells, is the perfect tool to decipher the effects of extreme and repetitive mechanical stress, such as cycles of gravity modification, on physiological elements. Such repetitive "traumatic" perturbations are suspected to be involved in the aging process by modifying regenerative and homeostatic behaviors of cells. With this device, we are able to gain new insights and understanding into human developmental biology and tissue physiopathology and the effects of external factors on the human body.

Presentation: Oral

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Noninvasive, *in-situ* bioluminescence sensing enables automated, real-time tracking of fluorophore concentration on-chip

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Over the past 30 years, microphysiological systems (MPS) have emerged as a robust alternative to address the technological gaps associated with current *in vitro* and *in vivo* options. Although recent advancements in MPS technology have demonstrated an extensive range of characterization methods on-chip, most approaches require human intervention for data acquisition and analysis, limiting device translation and feasibility in high-throughput applications [1].

Herein, we describe a novel organ-on-chip (OOC) platform that integrates automated, temporal bioluminescence sensing on-chip. The platform adapts a previously validated laser cut and assembly method to fabricate a multilayer OOC that features reversibly sealing ports for fiber optic cables [2,3]. Similar to fiber photometry, an optical setup delivers excitation light via fiber coupled LEDs and records fluorophore emission via CMOS cameras. A linked Arduino microcontroller automates image capture, permitting remote data acquisition and analysis. Diffusion of fluorescein dextran across a semipermeable polycarbonate membrane (0.4 mm pore size) was tracked remotely in the described OOC, recording fluorescent intensities within apical and basal compartments simultaneously. Fluorescent intensity was correlated to concentration via a standard curve, and relative membrane permeability under static and flow conditions was calculated. Comparison of relative permeabilities across dextran sizes (4 kDa, 70 kDa) elicits the expected relationship between molecular weight and relative permeability, offering support for the platform. Ongoing work is focused on applying this technology as a noninvasive analog to transepithelial electrical resistance in live cell culture. It is anticipated that the platform will provide real-time outputs of membrane permeability, allowing users to track cell health on-chip without perturbation. The platform may also be adapted to sense any fluorophore using appropriate dichroic mirrors and excitation/emission filters, broadening applications to luciferase assays, calcium sensing, and fluorescent protein expression. Overall, this technology is a small step toward addressing significant rate limiting steps of data acquisition and throughput for OOCs, with future work focused on closed-loop control and automated characterization of living cell culture conditions.

References

[1] Zhang, B. et al. (2018).
[2] Hosic, S. et al. (2021).
[3] Hosic, S. et al. (2019). U.S. Patent No. 20190083979

Presentation: Poster

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A photonic biosensor-integrated tissue chip platform for real-time sensing of secreted biomarkers

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Tissue chip (TC) devices seek to mimic human physiology on a small scale. They are intended to improve upon animal models in terms of reproducibility and human relevance, at a lower monetary and ethical cost. Virtually all TC systems are analyzed at an endpoint, leading to widespread recognition that new methods are needed to enable sensing of specific biomolecules in real time, as they are being produced by the cells. To address this need, we developed a two-channel microfluidic TC device with incorporated photonic biosensors situated in immediate proximity to the cells under study, which can measure the presence of specific analytes in real time. Using this novel platform, we've developed a better understanding of lung inflammatory response by measuring the time course of analyte secretion in a model bronchial epithelial barrier. Additionally, movement of exogenous analytes in the top channel through the tissue barrier after experimental barrier disruption was detected with the photonic sensors, and used as a surrogate marker of barrier breakdown. Finally, we have applied this model system to brain endothelial cells, beginning to build towards a model of the blood-brain barrier with applications in several disease models, including Parkinson's Disease. This represents an important step in the development of a high-dimensional data tissue chip.

Presentation: Poster

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Intelligent magneto-mechanical system to simulate physio- and pathologically relevant mechanical dynamics *in vitro*

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We present a novel approach to study the mechanobiological processes that occur during complex and dynamic mechanical scenarios. Biological cells and tissues are continuously subjected to mechanical stress and strain cues from their surrounding substrate. How these forces modulate cell and tissue behavior is a major question in mechanobiology. To conduct studies under controlled varying patho- and physiological strain scenarios, a new virtually-assisted experimental system is proposed allowing for non-invasive, dynamic and real-time control of complex deformation modes within the substrates [1]. This approach is based on the use of extremely soft magneto-active polymers that mimic the stiffness of the extracellular matrix (~1-10 kPa). The system enables the untethered control of biological substrates providing reversible mechanical changes and controlling heterogeneous patterns.

The design of the multifunctional cell substrate is conceived by integrating magneto-active domains in a functionally-graded fashion. To enable so, we have conceptualized a novel 4D printing approach that combines a hybrid theoretical-experimental framework [2]. This allows for optimal printability of time-dependent reactive inks using the direct ink writing technology (DIW). The complete method is implemented within an in-house printer and validated for different soft functional multimaterials.

The flexibility and robustness of the cell-actuation system, fed by advanced magneto-active substrates, is demonstrated under relevant scenarios. We addressed a major challenge in mechanobiology: to reproduce the mechanical dynamics of traumatic brain injury and brain stroke *in vitro*. By coupling our stimulation system to imaging and instrumentation devices we provide, for the first, temporal changes in morphology, local apparent stiffness and calcium dynamics in astrocytes. Overall, our new technology opens the way to understanding the mechanobiological processes that occur during complex and dynamic deformation states, such as in traumatic brain injury or pathological skin scarring.

This work was supported by the European Research Council Starting Grant nº 947723, project: 4D-BIOMAP.

References

[1] Moreno-Mateos, M. A. et al. (2022). Magneto-mechanical system to reproduce and quantify complex strain patterns in biological materials. *Appl Mater Today 27*, 101437.

[2] Lopez-Donaire, M. L. et al. (2022). Computationally guided DIW technology to enable robust printing of inks with evolving rheological properties. *Adv Mater Technol*, 2201707.

Presentation: Oral

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3D liver-on-chip with a perfusable physiologic-like vascular channel

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The recapitulation of 3-dimensional (3D) architecture of the liver, including its vasculature, is paramount for the generation of functional *in vitro* hepatic models. In recent years, advanced liver-on-chip (LoC) able to provide biochemical and physical stimulations to enhance liver-specific functionality have been developed [1]. However, some of them show physical barriers (e.g., membranes or scaffolds) between hepatocytes and endothelial cells and do not provide physiological stiffness values to the hepatocytes, thus poorly recapitulating the native hepatic *milieu*. Moreover, they fail to recapitulate the intrinsic circularity of vascular channels and physiological shear stresses, which are known to promote barrier functionality and cytoskeletal alignment of endothelial cells [2]. We aimed at developing novel LoC systems designed to generate vascularized and functional 3D hepatic microtissues without any physical separation between hepatocytes and endothelial cells. Our platforms allow the generation of vascularized and perfusable cylindrical channels inside low-volume (i.e., 1µL) chambers hosting primary human hepatocytes (PHH)-based 3D hepatic constructs. Additionally, in our models, endothelial cells (i.e., human umbilical vein endothelial cells (HUVEC)) are subjected to an *in vivo*-like shear stress, and hepatocytes can experience physiological liver extracellular matrix (ECM) stiffness. Two platforms have been developed (with and without the inclusion of an ECM layer mimicking the space of Disse) and validated for primary cell culture. Hepatic functionality was assessed (e.g., albumin production and enzymatic activity), showing that the recapitulation of the space of Disse enhances hepatic functionality compared to when hepatocytes and endothelial cells are in direct contact, thus better mimicking the *in vivo* conditions. Additionally, the platforms are compatible with the uBeat® technology [3], that provides microtissues with a controlled mechanical stimulation, which is amenable for integration within multi-organ settings where recapitulating liver functionality and metabolism is of fundamental importance for drug efficacy and safety applications.

References

[1] Polidoro, M. A., Ferrari, E. et al. (2021). *Liver Int 41*, 1744-1761.

[2] Polacheck, W. J., Kutys, M. L., Tefft, J. B. et al. (2019). *Nat Protoc 14*, 1425-1454.

[3] Marsano, A., Conficconi, C., Lemme, M. et al. (2016). *Lab Chip 16*, 599-610.

Presentation: Oral

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Microbiome characterization using marker-independent imaging for organ-on-a-chip applications

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The human microbiome plays a crucial role for human health and predisposition to a variety of diseases. Early identification of life-threatening pathogens as well as understanding their interactions with the microbiome will enable the development of novel treatments, potentially overcoming limitations by multi-resistant strains. However, static culture of nasal microbiome for *in vitro* testing remains challenging and conventional readouts to study host-microbiome interactions such as gene and protein expression requires time-consuming and destructive sample preparation.

Therefore, our aim was to combine a sophisticated OoC environment simulating the interface of the human nasal microbiome consisting of mucosal cells and multiple bacteria strains with non-invasive imaging techniques that enable *in situ* readouts at spatial resolution. Raman microspectroscopy generates molecule specific spectral fingerprints and thus enables marker-free identification and localization of cellular structures. Combined with machine learning approaches, Raman imaging was implemented to discriminate different bacteria strains and characterize their metabolites as well as the mucosal cells. Furthermore, to optimize in-chip imaging and decrease interferences of background signals from the polymer materials applied for chip fabrication different synthetic and biological membranes were tested.

The generated workflow, reference spectra and data-processing tools will now allow us for applying the nasal-microbiome-on-chip model to study infections and host-microbiome interactions in a spatial and time-resolved manner.

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In vitro models for the human placental barrier

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Cell cultures utilizing microfluidic devices can facilitate the formation of tissue-like cell structures from stem cells, which are expected to be useful for drug development. In drug development, it is critical to evaluate the extent to which target chemicals cross the human placental villi barrier and are transferred from the mother to the fetus to avoid adverse effects on the fetus. Such evaluations have been challenging to conduct using animal models due to the structural dissimilarities between the human and animal placentas. While some placental barrier models have been reported utilizing placental cell lines, the functions of these cells differ from those of *in vivo* placental cells. In this study, we show *in vitro* placental barrier models using a microfluidic device loaded with trophoblast stem cells (TS cells) [1] derived from the human placenta.

A polydimethylsiloxane device featuring upper and lower channels was fabricated to perfuse culture media. A permeable basement membrane composed of vitrified collagen was interposed between the upper and lower channels. TS cells were seeded into the microfluidic device, and culture media were perfused at a rate of 30 μL/h using a syringe pump. Following cell culture, cells were fixed with paraformaldehyde, stained with antibodies, and analyzed under fluorescence microscopy.

The perfusion of culture media enabled TS cells to differentiate into syncytiotrophoblast (ST) cells that function as a placental barrier. ST cells were formed via the fusion of TS cells. Notably, ST cells were scarcely observed in a microfluidic device lacking perfusion. Analysis with a confocal microscope revealed that the ST cells formed a single layer on undifferentiated cells, mimicking the structure of human placental villi.

In conclusion, this study demonstrates that ST cells can be formed under perfusion conditions within a microfluidic device. The morphology of the formed ST cells partially resembled that of the human chorionic villus. This device has potential utility as a valuable tool for assessing the transfer of chemicals to the fetus via the placenta in drug development.

Reference

[1] Okae, H. et al. (2018). Derivation of human trophoblast stem cells. *Cell Stem Cell 22*, 50-63.

Presentation: Oral

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A novel physiologically relevant tissue explant gut-on-a-chip model with an aerobic-anaerobic interface to study host-microbe interactions

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Objectives and study: The gut microbiome affects both intestinal and overall health, but studies on microbiome-related effects in the human gut are minimal due to the complexity and technical challenges of exposing intestinal epithelial cells, which need oxygen to survive, to gut microbiota that only survive in an oxygen-free environment. To tackle this, we developed an aerobic-anaerobic interface for our gut-on-a-chip model called the Intestinal Explant Barrier Chip (IEBC), allowing to study gut microbiota in an oxygen-free environment co-cultured with intestinal epithelial tissue to study host-microbe interactions.

Methods: The aerobic-anaerobic interface was established by connecting the apical recirculating flow of the IEBC to a conditioning chamber with anaerobic atmosphere, containing the medium reservoirs, sample inlets and oxygen sensors. *Ex vivo* gut colon tissue of human and porcine origin was incorporated into the IEBC and co-cultured with strict anaerobe *Bifidobacterium animalis* for 24 hours.

Results: Low oxygen levels (< 0.5%) in the apical medium confirmed that the aerobic-anaerobic interface was preserved. Tissue explants cultured in the aerobic-anaerobic interface showed proper tissue functionality (transcellular/paracellular transport > 2), intact tissue integrity (FITC-dextran 4000 leakage < 0.5%/h) and significant differences in microbial beta diversity compared to aerobic tissue conditions, with a higher abundance of e.g. bifidobacterium and lactobacillus species. Tissue functionality and integrity remained intact upon co-culture with *Bifidobacterium animalis*, for which growth and tissue-attachment were shown by targeted qPCR analysis.

Conclusions: Here, we successfully demonstrated the use of our novel aerobic-anaerobic interface for the IEBC with the co-culture of a strict anaerobe and human or porcine colon tissue explants. Next, we will use this novel platform to study the impact of the (specific strains of) gut microbiome on gut health and oral drug absorption.

Presentation: Poster

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Printing 3D anisotropic heart chamber scaffolds with fiber infused gel inks

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In the heart, cardiomyocytes and their ECM networks form hierarchically organized muscle tissues, allowing for excitation-contraction coupling and cyclic beating [1]. Likewise, engineered heart models used to study heart disease, or eventually as regenerative therapeutics require a similar ECM network to recapitulate the structure-function relationships of the heart [2]. Biomaterials such as collagen-derived gelatins are attractive materials for tissue engineering as they provide structural support for cell-matrix adhesion and tissue development. Particularly, 3D printing has been increasingly used for engineering those biomaterials to build 3D-shaped *in vitro* tissue models [3]. However, efforts to date have shown a limited ability to produce the microstructural features necessary to promote cellular self-assembly in 3D organ-shaped models due to the limited printing resolution. Here, we developed a 3D-printable gelatin fiber-infused hydrogel ink to 3D print heart chamber scaffolds that recapitulate the anisotropic electrophysiological function and anatomical structure of the heart. The addition of gelatin fibers allows for the precise printing of free-standing 3D structures without additional supporting materials by tailoring ink viscosities and sol-gel transition properties. Shear-induced alignment of fibers occurred by printing provides microscale geometric cues that promote self-assembly of cultured induced-pluripotent stem cell cardiomyocytes (iPSC-CM) into anisotropic functional tissues *in vitro*. The 3D printed microphysiological system of *in vitro* ventricle model exhibited biomimetic anisotropic electrophysiological and contractile properties.

References

[1] LeGrice, I., Pope, A. and Smaill, B. (2005). The architecture of the heart: myocyte organization and the cardiac extracellular matrix. In F. J. Villarreal, *Interstitial Fibrosis in Heart Failure.* Developments in Cardiovascular Medicine 253. New York: Springer.

[2] Thomas, D. et al. (2022). Cellular and engineered organoids for cardiovascular models. *Circ Res 130*, 1780-1802.

[3] Lee, A. et al. (2019). 3D bioprinting of collagen to rebuild components of the human heart. *Science 365*, 482-487.

Presentation: Oral

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Combining organ-on-a-chip and TK/TD modeling

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Despite the important contribution of animal experiments to the evaluation of drug safety in preclinical studies, ethical concerns and limited predictive power for clinical outcome drives the quest for alternative human-relevant models. Recent developments in organ-on-a-chip (OoC) microfluidic devices with interconnected tissue-engineered cultures have shown great promise to mimic human biology better than conventional systems.

Here, we present our exploratory study on the opportunities of combining OoC technologies with toxicokinetic and toxicodynamic (TK/TD) modeling to facilitate the prediction of the exposure-effect relationship and enhance safety assessment in preclinical studies for the endpoint liver toxicity.

We first experimentally validated the OoC system using a cell line-based gut model and qualified donors for the primary human liver model. We employed quantitative TK modeling to design experiments of (multiple) well-known tool compounds on gut-liver OoC systems with physiologically relevant exposure conditions. In addition, we used the similarity scaling approach to maximize clinical translatability of the data obtained from OoC systems.

The intrinsic pharmacokinetic parameters of all the studied tool compounds were then determined by performing a model-based analysis of the obtained data. The mathematical modeling can also address the challenges associated with OoC systems, including compound loss due to adsorption to the chip material.

To explore the potential of OoC systems in safety assessment, a TD model was added to the TK model to account for adverse effects occurring in drug induced liver injury (DILI). We showed that mechanistic TK/TD modeling of the processes taking place on a gut-liver OoC system can better predict the relationship between drug concentration and its hepatotoxic effect.

Mathematical integration of OoC experiments by means of modeling & simulation will increase the translatability of such innovative and promising preclinical methodologies and will thereby improve the safety assessments in preclinical development.

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Comparison of commercial NASH models as tools for pharmaceutical research and development

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Non-alcoholic steatohepatitis (NASH) embodies the most severe form of non-alcoholic fatty liver disease in the Western population. NASH develops as reversible hepatocellular steatosis (accumulation of neutral lipids in the liver), triggering inflammation and an excessive deposition of fibrillar extracellular matrix (ECM) (liver fibrosis). Chronic inflammatory and pro-fibrotic stimuli induce both morphological and functional impairment in the liver, ultimately resulting in liver cirrhosis and/or in hepatocellular carcinoma.

Much effort has been undertaken to develop effective therapeutics to treat NASH. In the past, disease understanding and evaluation of novel therapeutic approaches were derived mainly from animal studies. However, it has often been challenging to translate promising preclinical evidence into therapeutic success in patients. In recent years, advanced *in vitro* liver models, including 3D spheroid (Liver microtissues, LiMTs) and microfluidic (liver-on-a-chip) models, have emerged and paved the way to address more complex biological questions, such as NASH, with a potentially higher translational value.

Here we present data on the induction of NASH-like phenotypes in the LiMTs and liver-on-a-chip models, covering several important hallmarks. We further compared the transduction efficiency of adeno-associated virus AAV vectors between the control and NASH models. The prominent liver targeting AAV vector, AAV-DJ capsid encapsulating a mCherry reporter gene, was utilized. Both imaging and molecular analysis suggest that AAVs can be used as a gene therapy vector for NASH patients.

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Human atopic diseases on a chip: Developing an ex *vivo* drug discovery platform

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Background: Atopic diseases such as atopic dermatitis and allergic asthma are predominantly driven by T helper type 2 (Th2)-mediated inflammation. Thymic stromal lymphopoietin (TSLP) is a master regulator of Th2 inflammation, which is also significantly increased in atopic patients and hence considered a druggable target. In pre-clinical studies, mouse models are usually used for drug development. However, atopic diseases do not spontaneously develop in rodents. Furthermore, murine and human TSLP share poor homology, with no cross-reactivity between the species. There is no suitable animal model available that properly recapitulates human atopic diseases. Hence, there is a huge need for complex, human-based models to close this translational gap. Here, we aim to develop an atopic disease-on-a-chip model that can be used as a drug discovery platform.

Methods: In vitro 3D skin (healthy and diseased) and lung models were generated as described previously and mounted on a microfluidic multi-organ-on-a-chip (OOC) platform. Activated human CD4+ T cells were added to the microfluidic channel to mimic the human Th2 inflammatory condition. A novel anti-TSLP compound (BP79) and Tacrolimus were applied topically on the skin models to test drug efficacy. Characterizations were performed with ELISA, cytotoxicity assay, q-PCR, RNA sequencing, histology, and immunofluorescence microscopy.

Results: The models cultured on the OOC platform exhibited *in vivo* physiological characteristics such as morphology, histology, and protein expression. Topical application of BP79 on the skin resulted in significant down-regulation of key inflammation markers such as TSLP, periostin, IL-13, and IL-4, migration of CD4+ T cells in the skin and lung models, increased filaggrin expression, as well as anti-inflammatory effects in the lung model as determined by RNA-Seq analysis. Tacrolimus treatment also significantly suppressed IL-4 and IL-13 secretion but failed to reduce TSLP and periostin expression, CD4+T cell infiltration, or induce Filaggrin expression compared to BP79.

Conclusion: We have developed an organs-on-a-chip model that closely emulates human atopic diseases and has great translational potential as a drug discovery platform and beyond.

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Organotypic system for modeling developmental toxicity in testis

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A limitation in predictive toxicology is a lack of microphysiological models for the developing reproductive systems. Successful *in vitro* models of toxicity in male reproductive development need to recapitulate cell types and functional activities in neonatal testis. To address this, we developed an organotypic model of post-natal testicular development (testis coculture system, TCS) using primary neonatal rodent cells. The TCS incorporates 3-D co-culture of primary postnatal day five rat testis cells in a novel micro co-culture plate. Each culture is seeded with ~25k cells in Matrigel to promote formation of 3D structure. In pilot experiments, the TCS was maintained through 16 days in culture. Culture media was sampled for testosterone and RNA isolated for transcriptomics and RT-qPCR.

In the absence of exogenous hormones, testosterone production decreased over seven experimental days ($p < 0.001$), then stabilized and was maintained through experimental day 16. Transcriptomic data through nine days in culture showed expression of testis marker genes and increased expression of genes suggesting physiologically relevant testicular development: Igf1 [log fold-change (logFC) = 1.8, adjusted p-value (adj.p) = 0.001) and Nrg1 [logFC = 2.4, adj.p = 0.049). RT-qPCR data through 16 days in culture suggests physiologically relevant increases in Cyp19a1 (5.3-fold increase, $p = 0.02$) and Star (6.2-fold increase, $p = 0.02$). The TCS was exposed to follicle stimulating hormone (FSH) and luteinizing hormone (LH) at low (5 mIU/mL FSH and 50 mIU/mL LH) and high doses (20 mIU/mL FSH and 500 mIU/mL LH) to determine capacity for hormone induced testosterone production across the culture time. Hormone exposure increased testosterone production in the high (mean = 23.0 ng/mL, 95% CI: 15.3-30.7 ng/mL, p-value < 0.001) and low (mean = 8.2 ng/mL, 95% CI: 2.4-13.9 ng/mL, p-value < 0.01) experimental groups.

Our data supports that the TCS maintains major testis cell types, recapitulates physiologically relevant developmental trajectories, and is functionally responsive to exogenous hormone signaling. Ongoing studies with the TCS are investigating the effect of phthalates on inflammatory signaling and somatic cell development. Future directions include studying paracrine interaction between the TCS and the peripheral immune system through co-culture with monocytes, to mimic *in vivo* immune activation and recruitment.

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Influence of protein corona formation onto gold nanoparticles in a dynamic regime by microfluidic devices

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Is PC formation equal to dynamic and static conditions? A major number of the papers are related to the study of PC formation in static conditions, which is far from reality. In this sense, a central aspect that has been poorly studied is the nanoparticle-protein interaction within a dynamic environment caused by a flow. Microfluidic (MF) is a pioneering technique that allows analyzing and studying hydrodynamic regimes. Furthermore, it can emulate a natural environment to explore the nanoparticle-protein interaction by simulating a PC formation in the bloodstream. Therefore, for this reason, it is vital to consider the potential information that these experiments could provide to mimic the natural conditions that nanoparticles could face in the bloodstream.

With this question in mind, GNPs will be characterized to determine the interaction with plasma proteins (fibrinogen, albumin, and myoglobin) under a MF regime. In this way, it will be possible to study the PC formation, emulating natural conditions and comparing their behavior with macroscale conditions. Soft lithography will fabricate the previously designed microchips in polydimethylsiloxane (PDMS). The PC formation will be physiochemically characterized.

This work has an important novelty and is framed in alignment with studies that are not being carried out in this country. Our results could provide valuable information for the validation of NPs for future treatments, for example, without having to use animal models

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Developing perfusable choriocapillaris for an outer retinal blood barrier-on-a-chip

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Dysfunction of the outer retinal blood barrier (ORBB), composed of the retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris (CC), is implicated in age-related macular degeneration (AMD), a leading cause for central vision loss. The mechanisms of AMD remain poorly understood, in part due to the limitations of animal models. Advances in tissue engineering and organ-on-a-chip technology have catalyzed the rise of microphysiological systems (MPS) as an alternative to preclinical testing [1]. Previously, we modeled CC vasculature by encapsulating endothelial cells (ECs) within a poly(ethylene glycol) (PEG) hydrogel-based engineered extracellular matrix (eECM) [2]. While our model supports development of CC-like vascular networks, it lacks perfusion. Current MPS for vascularized tissues like the oRBB forego perfusion or rely on EC-lined fluidic channels. These MPS lack control over key elements of the vascular microenvironment, including microfluidic and ECM physicochemical cues [3]. To address this gap, we have created the *Outer Retinal Blood Barrier-on-a-Chip (ORBB-C)*, which exploits microfluidics and a tunable PEG eECM to control these features of microvascular development. We hypothesize that eECM physicochemical and microfluidic cues can be combinatorially modulated to guide the development of perfusable, CC-specific microvasculature. Human umbilical vein endothelial cells (HUVECs) were co-encapsulated with human mesenchymal stem cells (hMSCs) in PEG eECM hydrogels. Flow cytometry and immunofluorescent imaging were used to interrogate the effects of eECM physicochemical cues (i.e., matrix metalloproteinase (MMP) degradability, adhesive peptide concentration, and modulus) and microfluidic pressure gradient on angioarchitecture (vessel density, average vessel diameter, total vessel length) and CC-specific differentiation. Our initial findings show that HUVECs form networks with significantly higher average vessel length and branch density when encapsulated in PEG hydrogels with lower elastic modulus and high MMP-degradability (defined by the catalytic efficiency k_{cat}/K_m). These results indicate that the physicochemical properties of PEG eECM can be manipulated to recapitulate specific microvascular tissues like the CC. Ongoing efforts are focused on determining if physiological flow establishes perfusibility and further improves structural properties of self-assembling microvasculature.

References

[1] Shirure et al. (2021). *Annu Rev Biomed Eng*.
[2] Manian et al. (2021). *Cell Stem Cell*.
[3] Ewald et al. (2021). *Lab Chip*.

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Photopatterned synthetic hydrogels for perfusable gut-on-a-chip systems

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Intestinal bowel disease (IBD) is a chronic immune-mediated disorder characterized by chronic inflammation of the gastrointestinal tract, comprising Crohn's disease and ulcerative colitis. An estimated 3.1 million adults in the US have been diagnosed with IBD, with annual costs exceeding $25 billion (Siddharth et al., 2022). The unknown etiology of IBD, along with its heterogeneous and multifactorial nature, make essential the development of relevant and functional model platforms that enable the fundamental understanding of the disorder. Human intestinal organoids (HIOs) have enormous potential for IBD modeling, but their derivation in static three-dimensional matrices without perfusion can restrict their development and functionality. Currently, the fabrication of perfusable gut-on-a-chip platforms that include hydrogel matrices for HIOs culture, involve painstaking, time-consuming, and specific laser-based methodologies limited to natural matrices (Mitrofanova et al., 2022). Here, we engineered a rapid and facile methodology to generate complex hydrogel structures to use in gut-on-a-chip models for IBD diagnostic, modeling, and drug screening applications. Our strategy employs ultraviolet light-triggered polymerization of synthetic polyethylene glycol hydrogels and a photomask for the generation of complex patterns, including perfusable channels. This approach reduces preparation times from several hours to seconds and requires simple instrumentation. Moreover, the ability to use synthetic hydrogels provides tremendous flexibility and lowers regulatory burdens (U.S. Patent Application 63/273,224). Our optimized hydrogel formulation provided a reduced swelling (< 10%), which was essential to maintain an excellent shape fidelity (~85%) of the developed features in the gut-on-a-chip device. Single cells obtained after disassociation of mature HIOs were seeded in the central lumen and cultured with and without media perfusion. After 3 days, perfused devices showed a lu-

men surface coverage of 69 ± 0.3% with a cell viability of 96 ± 2%, in comparison to non-perfused devices that showed 3 ± 1% and 31 ± 3% of lumen coverage and cell viability, respectively. Moreover, intestinal markers (e.g., CDX2, E-cadherin) were observed in the cultured cells in the devices, demonstrating that our gut-on-a-chip systems are suitable and accessible platforms for the development of more physiologically relevant intestinal organoids. Further experiments will focus on intestinal functional studies as well as long-term culture models.

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Automation of multi-organ-chip assays

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With the approval of the FDA Modernization Act 2.0 the requirement to use animal testing for drug development has finally been eliminated – paving the way for innovative animal-free technologies like Multi-Organ-Chips. However, there are still major challenges ahead. Currently, the full potential of MPS cannot yet be fully exploited. The high complexity of MPS-based assays leads to a high amount of manual work in the execution and analysis of the assays. This problem can be solved by automating the execution of the assays. Here we present the HUMIMIC AutoLab, a newly developed system for full automation of complex MPS based assays. The system has its own incubation system and brightfield- and fluorescence microscope. A class II safety cabinet allows sterile handling. The built-in 4°C refrigerator allows the system to operate for up to four days without user interaction. The HUMIMIC AutoLab can culture up to 24 MOC's simultaneously. Media exchange, sampling, substance application, microscopy and more are automatically performed.

Several assays were performed to test the system. For example, the system demonstrated the ability to dynamically culture a MatTek EpiIntestinal™ model in combination with a HepaRG-based spheroid model in a HUMIMIC Chip2, in a 14-day experiment. In a PB/PK experiment, it was shown that the HUMIMIC AutoLab can run hourly substance applications and media changes to mimic physiological substance profiles. During the execution of the assays, it was shown that the automatic sampling and the integrated microscope can generate significantly more data points than in a manual experiment. Combining this data with deep learning techniques in the next step, more readouts and analysis could be generated or performed without additional effort.

Based on the HUMIMIC AutoLab, it can be shown that in the future MPS based assays can be performed with a higher throughput with less manual effort. MPS-Assays performed with automated systems lead to a higher standardization and reproducibility, high-content data and the possibility for AI-based analysis. Together, these are important factors in advancing regulatory acceptance and industry adoption of MPS-based assays.

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Real-time monitoring of the effects of vasculature in a tumor microenvironment

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The tumor vasculature constitutes a key aspect of the tumor microenvironment (TME), which can significantly impact the behavior and therapeutic resistance of the tumor. However, the precise effects of vasculatures on tumor activities are not fully understood. Microphysiological systems (MPSs) have emerged as powerful tools to study tumor vasculature *in vitro*, as they can reproduce its dynamic nature, including the luminal and interstitial flows. In this presentation, we show a three-dimensional (3D) tumor model with a perfusable vasculature [1,2] and a vasculature model that reproduces the transition from endothelial to mesenchymal (EndoMT) phenotype [3], in which endothelial cells lose their endothelial markers for mesenchymal markers.

3D tumor models (MCF-7 spheroids or patient-derived colorectal cancer organoids) in microfluidic devices were vascularized in angiogenic or vasculogenic manners. Briefly, a tumor model was embedded in fibrin-collagen gel and cocultured with endothelial cells at the center of a microfluidic device comprising five or three microchannels. The cell proliferation and their metabolic activities were evaluated using immunohistological and electrochemical methods. In addition, a vasculature model simulating the endothelial-to-mesenchymal transition (EndoMT) was fabricated using mouse liver sinusoidal endothelial cells (mLSECs).

We found that tumor vasculature enhanced tumor proliferation by immunostaining of Ki67 and ss-DNA. Furthermore, electrochemical sensors successfully detected the change in oxygen metabolism of the tumor model in a real-time manner. For the EndoMT model in a microfluidic device, mLSECs formed continuous vasculatures with luminal structures. However, the diameter of the mLSEC vasculature increased in a time-dependent manner, resulting in a single hollow vasculature within the channel. For the robust EndoMT model, optimizing culture conditions for mLSEC is the future issue.

In summary, we have shown the significant effects of tumor vasculature on 3D tumor models at the level of cell proliferation and oxygen metabolism. The use of microphysiological systems represents a crucial step in uncovering the mechanisms of tumor vasculatures, including the endothelial-to-mesenchymal transition (EndoMT).

References

[1] Nashimoto, Y. et al. (2020). *Biomaterials 229*, 119547.
[2] Nashimoto, Y. et al. (2023). *Biosens Bioeletron 219*, 114808.
[3] Yoshimitsu, Y. et al. (2022). *Inflamm Regen 42*, 9.

Presentation: Poster

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Development of evaluation methods of "points to consider" for industrial implementation of MPS

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MPS have attracted attention as novel *in vitro* culture method for evaluating safety and efficacy in new drug development. By culturing cells in a limited space, MPS can mimic the flow of blood in tissues *in vitro* and elucidate the mechanical effects of blood flow on cells as well as the effects of mass transfer, such as nutrients, metabolic waste products, and drugs. However, due to the unique nature of the culture environment, it has become clear that there are "points to consider" that differ from conventional culture methods in order to maintain healthy cell function. In Japan, the AMED-MPS project, led by AMED (Japan Agency for Medical Research and Development), are developing methods to evaluate these "points to consider" in terms of cell function, cell adhesion, and perfusion conditions [1]. The cellular functions of hepatocytes of different origins were compared and differences in drug-metabolizing activity were reported [2]. We also have examined cell adhesion and oxygen supply due to the limited space of cell culture in MPS, and clarified points to be considered. Attachment of cells differed depending on the materials used for MPS. The laminar flow of medium caused the gradient of dissolved oxygen concentration from the inlet to the outlet of culture chamber and caused the difference of cell viability and mitochondrial functions depending to their position in the chamber. These results are important to consider the cell propagation and maintenance in MPS. In this presentation, we will report these results and discuss "points to consider" for industrial implementation of MPS.

References

[1] Ishida, S. (2021). Research and development of microphysiological systems in Japan supported by the AMED-MPS project. *Front Toxicol*. doi:10.3389/ftox.2021.657765
[2] Horiuchi, S., Kuroda, Y., Komizu, Y. et al. (2023). Consideration of commercially available hepatocytes as cell sources for liver-microphysiological systems by comparing liver characteristics. *Pharmaceutics 2023*. doi:10.3390/pharmaceutics 15010055

Presentation: Oral

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Elucidating normal liver-small intestine interactions in terms of drug metabolism using on-chip perfused and direct oxygenated MPS

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Organ interactions affect various phenomena such as drug metabolism and systemic response. Especially between the small intestine and liver where drug absorption and metabolism take place with the presence of enterohepatic circulation. Modulation of drug metabolizing enzyme (CYPs) activity when hepatocytes were co-cultured with small intestine cells has been demonstrated *in vitro*. However, the mechanism behind it is still unclear. Understanding such mechanisms could help in creating better model that is metabolically closer to human body.

In order to study the pure organ interactions between the liver and small intestine, healthy normal cells and suitable microenvironment to support their functions are essential. We used human iPS-derived intestine epithelial cells (hiPS-intestine) and primary human hepatocytes harvested from chimeric mouse (PXB-cells), cocultured in a MPS equipped with on-chip pumping mechanism using a micro-stirrer and direct oxygenation using oxygen permeable membrane made of PMP.

Increase in organ-specific functions was observed. Both albumin secretion and TEER measurements were higher in culture using MPS compared to conventional method. This shows that on-chip perfusion and direct oxygenation provides better *in vitro* microenvironment that makes the cells maintain functionality comparable to that *in vivo*. The activity of CYP enzymes in the PXB-cells was also measured and higher activity was observed when the PXB-cells were cocultured with hiPS-intestine cells. This indicates that the presence of organ interactions affects the drug metabolism in the liver.

We examined possible organ interactions mechanisms by RNA-seq. The results showed that direct oxygenation and perfusion gives a more pronounced coculture effect. Further study using GO analysis revealed that arachidonic acid (ARA) metabolism might play an important role in the modulation of PXB-cell's CYP activity during coculture. Indeed, addition of ARA to PXB-cells culture increased activity of some CYP enzymes. We suspect that ARA molecules from the hiPS-intestine initiates signaling cascade that results in higher CYP activity of the PXB-cells. Thus, our study shows the potential of MPS combined with healthy and normal cell sources as a tool in examining pure organ interactions.

Presentation: Poster

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Utilizing human cardiac organoids as multipurpose tool to study cardiac pathophysiology *in vitro*

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The prevalence of cardiovascular diseases will continue to rise in the coming decades. Therefore, to sufficiently recapitulate the (patho-) physiology of the human heart there is an urgent need for sustainable and complex *in vitro* models. This is especially important regarding drug discovery and development, leading to more reliable *in vitro* test systems and the identification of safe and potent drug candidates for further *in vivo* studies. Here, three-dimensional cell culture approaches as organoids aim to bridge the gap between common two-dimensional *in vitro* systems and *in vivo* studies.

Based on previously published protocols [1,2], we have established a platform for human cardiac organoids (hCOs) consisting of hiPSC-derived cardiomyocytes (CMs), cardiac fibroblasts, endothelial cells and adipose-derived stem cells. We demonstrated that these cell types self-assemble into an organized 3D structure where the CMs arranged at the outer layer of the hCOs and the non-CMs tend to assemble within the organoid's core. The spontaneous contracting hCOs were treated with the specific myosin-7 inhibitor mavacamten, leading to a decreased contractility and increased contractility when treated with adrenergic agonist phenylephrin-Isoprenaline. Apart from this, we utilized these hCOs to model myocardial infarctions induced by a hypoxic environment that led to impaired calcium handling and changes in fibrosis-related gene expression. Further, upon hypertrophic stimulation of healthy hCOs a hypertrophic phenotype was detected as ANP and BNP expressions were upregulated. In future studies, we aim to combine this 3D platform with our hypertrophic cardiomyopathy patient-derived iPSC-CMs. The organoids can then be used for molecular characterization of the disease and serve as a drug discovery platform. In summary, these initial data suggest the versatility of the multicellular hCOs to model and study cardiac diseases.

References

[1] Richards, D. J., Coyle, R. C., Tan, Y. et al. (2017). Inspiration from heart development: biomimetic development of functional human cardiac organoids. *Biomaterials 142*, 112-123.

[2] Richards, D. J., Li, Y., Kerr, C. M. et al. (2020). Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity. *Nat Biomed Eng 4*, 446-462.

Presentation: Poster

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A human multi-organ chip combining human liver and blood-brain barrier to predict drug pharmacokinetics and metabolite distribution

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In vitro models are indispensable tools in early drug screening. However, most *in vitro* models are not capable of recapitulating multi-organ interactions. The advent of microphysiological systems (MPS) allows scientists to recapitulate human organs *in vi-*

tro with an increased physiological relevance and allows the connection of multiple organ models in one circuit. Here we report the setup of a multi-organ chip which combines human liver and blood-brain barrier (BBB) *in vitro* models. First, we describe the differentiation of brain endothelial cells from human induced pluripotent stem cells. We show that the generated BBB model forms a tight barrier. Next, we demonstrate that the model's membrane integrity and gene expression is comparable when cultured in endothelial media or liver media. Finally, we interconnect the human BBB model with a previously published liver spheroid model in a multi-organ chip [1].

As a proof of concept, we follow the pharmacokinetics (PK) of midazolam and rosiglitazone in the multi-organ chip model in the surrogate "plasma" and "brain" compartment for 4 days. The concentration of both drugs can be described in a mathematical model, which can be used to estimate drug clearance and brain permeability in the system. Finally, similar to the *in vivo* situation we observe that the BBB repels the entry of several drug metabolites into the brain compartment. In conclusion, we show the setup of a liver-BBB multi-organ chip model that can be used to predict various PK parameters. The model also allows additional assessment of drug metabolite distribution into the brain compartment, which is important for the evaluation of efficacy and safety of drug candidates in the brain.

Reference

[1] Cox, B., Barton, P., Class, R. et al. (2022). Setup of human liver-chips integrating 3D models, microwells and a standardized microfluidic platform as proof-of-concept study to support drug evaluation. *Biomater Biosyst 7*, 100054.

Presentation: Poster

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Round lumen-based microfluidic devices for modelling cancer metastasis

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Most cancer related deaths are not caused directly by the primary tumor, but by secondary tumors formed through metastasis to other organs [1]. Metastasis is a complex cascade that we poorly understand due to the limitations of current *in vitro* models. Hence, we focus on modeling cancer metastasis-on-chip via introducing the relevant physiological factors in tumor microenvironment (TME). To bring current chip devices one step closer to the physiological morphology of (micro)vessels and breast-duct in the TME, we fabricate round luminal channels. For this, we exploit different techniques, such as 3D sugar-printing, needle casting and femtosecond laser (FSL) machining to form lumens either in hydrogel or PDMS.

In the 3D sugar-printing technique [2], sugar glass is printed as fibers and cast in surrounding material (nonpolymerized hydrogel, or synthetic polymers); the sugar glass is selectively dissolved after polymerization of surrounding material, which results in interconnected perfusable round lumens. We also achieve this with micro-needle technique, in which needle removal after hydrogel polymerization leaves behind a round lumen. For the fabrication of smaller dimension interconnected lumens, we utilize the FSL technique. A scanning laser beam induces fine modified patterns within the material. Upon subsequent wet chemical etching, the modified patterns are removed, leaving behind round lumens.

When the lumens are seeded with endothelial cells, they form a (micro)vasculature. Combined with a neighboring lumen for cancer cell culture, the process of cancer invasion, migration through extracellular matrix, and intravasation can be studied. Using these luminal channels, we could mimic various sizes (150-700 mm) of breast duct, blood, and lymphatic vessels-on-chip, in static and perfusion conditions. Cell-cell tight junctions were present, as a basis for further study of the metastatic cascade. We are currently generating human B-cell lymphoma-on-chip as an innovative model to study the effect of fibroblastic reticular cells in malignant B-cells dissemination (de Winde et al., unpublished). In future work, we will incorporate vessels in cancer-on-chip models to study metastasis.

References

[1] Chaffer, C. L. and Weinberg, R. A. (2011). A perspective on cancer cell metastasis. *Science 331*, 1559-1564.

[2] Pollet et al. (2020). 3D sugar printing of networks mimicking the vasculature. *Micromachines 11*, 43.

Presentation: Poster

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A 3D-bioprinted microfluidic model of human glioblastoma for investigating tumor heterogeneity and drug resistance

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Glioblastoma multiform (GBM) is the most aggressive form of primary brain cancer, with a median survival rate of only 15 months. Despite the extensive research, the complex mechanisms underlying GBM, including its intra/inter-tumoral heterogeneity and the presence of glioblastoma stem cells (GSCs), have made it difficult to develop effective treatments. Rodents have been widely used as preclinical models to study GBM, but their relevance to human biology is questionable. Microphysiological systems (MPSs) have recently emerged as a valuable alternative to animal models and traditional cell culture methods. In this study, we present a next-generation GBM model that utilizes a combination of industrial 3D printing and bioprinting to create a reliable and scalable replica of human glioblastoma. Bioprinting is used to generate a blood-vessel-like structure lined with primary human brain microvascular endothelial cells (HBMEC) and surrounded by primary human astrocytes and/or human glioblastoma cancer cells (U87). When perfused with a cell culture medium at physiological pressure, this 3D-bioprinted system allows for an accurate representation of the flow of interstitial fluid, simulating the continuous flow through the blood vessels. Our results demonstrate that interstitial fluid flow is a key factor that regulates the expression of cancer stem cell markers CD133 and Nestin. Co-culture of cancer and primary cells also resulted in a reduced vascular barrier function and increased levels of pro-inflammatory cytokines such as IL8, MCP1, and IL6 that are relevant for GBM tumorigenesis. Finally, we demonstrate that perivascular stem-like cells present higher tolerance to drug treatment when compared to cancer cells detected in the periphery of the bioprinted model, supporting the hypothesis that the perivascular space plays a key role in drug resistance of GBM. These findings demonstrate the potential utility of this model in simulating the complex and heterogeneous nature of GBM. The ability to recreate the perivascular niche, the continuous flow of interstitial fluid, and the co-culture of multiple cell types, all together support the model's ability to replicate the complexities of GBM and its application in drug efficacy and toxicity testing as well as personalized therapy development in the future.

Presentation: Poster

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Development of electrochemical sensors to measure glutamate kinetics *in vitro*

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Glutamate is an abundant excitatory neurotransmitter in the central nervous system (CNS) and plays a key role in fundamental brain functions including synaptic plasticity, formation of neuronal networks and repair. Changes in glutamate homeostasis can result in neuronal death via excitotoxicity. Research suggests that glutamate levels are altered in patients with Alzheimer's Disease. Environmental exposures can (in)directly alter glutamate homeostasis. To better understand glutamate kinetics, we developed a microfluidic platform containing electrochemical biosensors for glutamate, compatible with human induced pluripotent stem cell (hiPSC)-derived glutamatergic neurons. The biosensors featured 10-channel high-throughput read-out and real time measurement for multiple hours with a 2 Hz sample frequency. We used photolithography to make platinum microelectrodes on a 175-µm thick glass substrate. Then, we electrodeposited a poly(meta-phenylenediamine) perm-selective membrane and drop-casted L-glutamate oxidase hydrogel onto the electrodes. Microchannels were created with laser cut pressure sensitive adhesive and sealed with a PDMS slab with inlet and outlet holes. We investigated the compatibility of the developed sensors with different types of culture media, buffers and coatings and found that sensors performed best in BrainPhys based media or PBS and found sensors to be compatible with Matrigel coating. We then investigated function and sensitivity of the biosensors. Media spiked with 60 mM L-glutamic acid was added to the cells and collected in 10 min intervals over a 60 min period. Glutamate levels in media samples were measured using the biosensors and compared to data obtained using a commercially available colorimetric kit, resulting in comparable outcomes. We assessed compatibility of the biosensors with our glutamatergic neurons and found that 2 days after plating, cells started to form networks, with clear solid network structures visible after 1 week in culture. Media spiked with 60 mM L-glutamic acid was added to the cells and uptake was measured as decreased extracellular levels. Cells rapidly restored glutamate homeostasis. Future efforts will focus on measuring glutamate release from the cells and changes in glutamate homeostasis resulting from exposure to environmental chemicals. Additionally, we will multiplex these sensors with optical sensors, creating a multi-modal platform with combined epigenetic, intra, and extra-cellular read-outs.

Presentation: Poster

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Development of a three-dimensional blood-brain barrier microphysiological system with perfusable capillary opening structures for drug transport assays

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Microphysiological systems (MPS) have rapidly evolved as promising *in vitro* tools to emulate human physiology by closely reproducing key biological processes and functions of organ/tissue. The blood-brain barrier (BBB), a highly selective barrier between the circulatory system and the central nervous system, is composed of brain microvascular endothelial cells (BMEC), pericytes and astrocytes. Current BBB MPS fail to reproduce the human structural complexity of the brain microvasculature, and thus their functions are not enough for drug assessments. The transferrin receptor (TfR), one of the main routes for iron transport in the brain, shows great potential for drug transport due to its high expression level by both brain BMEC and brain cancer cells, but is poorly investigated in the current BBB MPS. Therefore, a BBB MPS displaying higher discrimination of candidate molecules based on their TfR-mediated transcytosis efficiency is expected to improve the sensitivity of the drug screening assays.

We developed a 3D self-assembled microvascular network formed by human BMEC, pericytes and astrocytes with perfusable capillary opening structures on the bottom of the fibrin gel [1], as originally reported for 3D blood-/lymph-capillary networks [2]. It demonstrated size-selective permeation of different molecular weights of dextran, which highly correlated ($R^2 = 0.973$) with the permeability values found with *in vivo* rodent brain. The presence and functionality of the TfR-mediated transcytosis was also confirmed by permeability assays. Efficient permeability coefficient (P_e) of transportable anti-TfR antibody (MEM-189) was about 4.77×10^{-6} cm/s, being seven-fold higher than those of isotype antibody (IgG1) and low transportable anti-TfR antibody (13E4) which are respectively 6.83×10^{-7} and 6.12×10^{-7} cm/s. These results suggest our model displayed a better capacity to discriminate antibodies based on their TfR-mediated permeation than previously reported [3]. This BBB MPS could be a valuable tool for the screening of therapeutics that can be transported across the BBB, including those using TfR-mediated transcytosis.

References

[1] Piantino, M. et al. (2022). *Mater Today Bio 15*, 100324.
[2] Hikimoto, D. et al. (2016). *Adv Healthc Mater 15*, 1969.
[3] Wevers, N. R. et al. (2018). *Fluids Barriers CNS 15*, 23.

Presentation: Oral

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A microphysiological system to investigate cell death pathways in inflammatory bowel disease for drug discovery and validation

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Cell death pathways play a crucial role in the pathogenesis of inflammatory bowel diseases (IBD), and the understanding of these pathways has led to the development of targeted therapies. Intestinal epithelial cell (IEC) death leads to barrier dysfunction and inflammation. Restoration and maintenance of the epithelial barrier or epithelial repair is a key therapeutic goal for new IBD therapeutics. Here we demonstrate a tiered pharmacology approach that compares barrier function across 3D colon organoids, 2D cell line based epithelial monolayers, and a microphysiological system (MPS) in response to known inducers of various cell death pathways.

Conditions to induce apoptosis and necroptosis were first established in 3D colonic organoids. Treatment of colonic organoids with apoptosis and necroptosis inducing cocktails resulted in a significant loss of cell viability. These conditions also induced barrier disruption in a 2D monolayer on transwell as measured by TEER and Lucifer yellow dye permeability. Therapeutic intervention, including Jak inhibition, revealed key differences in the ability of therapeutics to block pathway specific barrier disruption in both 2D and 3D models.

To enable the monitoring of cell death and permeability in real time, we next measured the impact of apoptosis and necroptosis cocktails in the Mimetas OrganoReady® Caco-2 based MPS plate. In this model, IFNγ and TNFα combined caused a 1.75-fold higher barrier permeability without inducing cell death. While a necroptosis inducing cocktail caused a 2.3-fold increase in cell death compared to vehicle which led to a 3.5-fold higher barrier permeability. A significant increase in the number of cells positive for phosphorylated mixed lineage kinase domain-like (MLKL) protein, a marker for necroptotic cell death, compared to controls was observed. Tight junctions are also heavily disrupted in the regions with abundant necroptotic clusters of IECs, confirmed by immunofluorescent (IF) staining of ZO-1. Work is ongoing to incorporate immune cells to induce barrier damage in place of recombinant factors. By adding physiological complexity, the MPS model has the potential to significantly improve IBD drug discovery.

Disclosures: All authors are employees of AbbVie. Opinion on the technology platforms is an opinion of the presenters, AbbVie takes no position on the technologies discussed.

Presentation: Oral

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Pancreas-liver *in vitro* and *in silico* hybrid model for human diabetic glucose dysregulation

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The growing epidemic of type 2 diabetes (T2D) is one of the major medical challenges today. T2D is characterized by hyperglycemia which is caused by dysfunctional communication between several glucose-regulating organs. As T2D is a multi-organ disease, preclinical studies of disease progression mechanisms are currently only possible in animal models. However, animal models used in diabetes research are genetically and physiologically different from humans leading to inaccurate translation [1].

Since the pancreas and the liver are central organs in blood glucose regulation, we and others have shown that coupling of pancreatic islets and liver spheroids on-chip can recapitulate human-relevant pancreas-liver axis [2,4]. Here, we further developed the model [2,4] for investigating diabetic glucose dysregulation on-chip. Due to the complex and dynamic nature of organ crosstalk, we combined the *in vitro* model with *in silico* modelling for hypothesis testing, data analysis, and informed decision-making. We investigated two medium supplements, glucose and cortisone, for their suspected influence on insulin sensitivity and beta-cell function.

The diseased pancreas-liver MPS displayed beta-cell dysfunction, steatosis, elevated ketone body secretion, increased glycogen storage, and upregulated gluconeogenic machinery in the liver compartment. In turn, physiological cortisone concentration and normoglycemia maintained glucose tolerance and stable liver and beta-cell functions. We also showed that IL-1R2, secreted from the liver compartment, can modulate islet proliferation. These findings did not only confirm that the liver and pancreas compartments exhibit disease-relevant cross talk on-chip but also further amplifies that the described model can be used to study new targets and therapies for diabetic patients. This method was evaluated for repeatability in two laboratories and was effective in multiple pancreatic islet donors.

Together, the pancreas-liver *in vitro* and *in silico* hybrid model for glucose dysregulation enables diabetes research in a human-based preclinical system. The model should facilitate drug discovery by serving as a platform for studies on disease mechanisms, target identification, and candidate drug evaluation.

References

[1] Chandrasekera, P. C. and Pippin, J. J. (2014). *ALTEX 31*, 157-176.
[2] Bauer, S. et al. (2017). *Sci Rep 7*, 14620.
[3] Tao, T. et al. (2022). *Adv Sci 9*, e2103495.
[4] Casas, B. et al. (2022). *PLoS Comput Biol 18*, e1010587.

Presentation: Oral

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Apically applied shear stresses impact the rheotactic behavior, physical forces, and transcriptomic profile of three different endothelial cell types

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Endothelial cells (ECs) inside blood vessels form a single cell monolayer that acts as a protective barrier against different harms, including pathogens. ECs are highly mechanosensitive and respond to a variety of physical cues originating from their extracellular environment, including changes in the blood flow over their surface. Flow shear stresses (SS) and shear stress gradients (SSG) vary in space, time and (patho)physiological conditions (e.g., arteriosclerosis, hypertension). Although EC responses to variations in SS magnitude have been studied to some extent, how SSGs modulate EC barrier function by impacting EC kinematics (i.e., speed, collective migration) and dynamics (i.e., traction and monolayer forces) is relatively less understood. Whether such effects are universal or cell type specific is also unclear.

Using a live-cell imaging-compatible impinging flow device, we found that exposure of three different EC types to flow led to an immediate, sustained and reversible 2-fold decrease in EC mi-

gration speed. Two out of the three EC types exposed to flow also increased directional collective movement against the flow direction. Traction force and monolayer stress microscopy analysis revealed a 50% increase in the traction forces ECs exert on their matrix and a 20% decrease in monolayer stresses – a proxy for the EC barrier integrity – after 20 h of flow exposure. This is consistent with the idea that enhanced actomyosin contractility and focal adhesion organization power the movement of ECs against the flow gradient, which we confirmed by pharmacological treatment. To identify the concurrent changes in biochemical signaling, we performed RNA sequencing in all EC types exposed or not to flow for 20 h and found that all three cell types upregulated TGF-b1 or genes related to it. We are currently investigating whether this is the common denominator regulating the increase in EC traction forces and the corresponding decrease in monolayer stresses during flow.

Collectively, our work suggests that flow exposure drives a dramatic reprograming in EC signaling and biomechanics, which is partly conserved across different types of ECs.

References

[1] Ostrowski, M. A. et al. (2016). *Ann Biomed Eng 44*, 2261-2272.
[2] Angelini, T. E et al. (2010). *Phys Rev Lett 104*, 168104.

Presentation: Poster

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Evaluation of metastatic tumor migration and invasion of secondary sites using a vascularized tumor-on-chip model

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Modeling the microenvironment of primary and secondary tumor sites is crucial for advancing treatment options for metastatic cancer. There are currently no 3D tumor models that mimic the *in vivo* vascular geometry and tumor microenvironment for monitoring extravasation, tissue invasion, and colonization of secondary sites by metastatic tumors. We report the development of a 3D tumor model with primary and secondary tumor sites, where metastatic breast cancer cells can escape the primary site, enter the circulation, adhere to the vascular endothelium and invade distant tissues. Synthetic Tumor Networks comprising primary and secondary tumor sites were developed using *in vivo* images and fabricated using soft lithography. Primary vascular endothelial cells were cultured in the vascular channels. Either a GFP-labeled metastatic human breast cancer cell line (MDA-MB-231/GFP), or a nonmetastatic breast cancer cell line (MCF-7/GFP), was cultured in the primary tumor site in a 3D environment using a hydrogel. The vascular networks were perfused with endothelial cell media under physiological fluid flow conditions. Real-time monitoring of cellular growth, intravasation, invasion, and extravasation were performed using fluorescence microscopy over a four-week period. Metastatic MDA-MB-231/GFP breast cancer cells proliferated rapidly in the primary tumor site in contrast to non-metastatic MCF-7. Tumor cells were found to initiate a metastatic cascade as early as 7-10 days following growth in the primary site, leading to intravasation into the vascular channels, adhesion to the endothelium and extravasation into the secondary site. Significant differences were observed in tumor behavior based on flow profiles and morphology of the vascular networks. These results show how the 3D vascularized model can be used for modeling tumor metastasis by mimicking the *in vivo* microenvironment of vascularized solid tumors. This Vascularized Tumor-on Chip model can be used to investigate tumor-endothelial cell interactions using a combination of real-time imaging techniques and screening of targeted therapeutics that disrupt tumor metastasis.

Presentation: Poster

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Pillar and perfusion plate platform for dynamic human organoid culture and analysis

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Several three-dimensional (3D) cell culture platforms have been developed, including ultra-low attachment well plates, Transwell inserts, hanging droplet plates, and microfluidic devices. However, these platforms are relatively low throughput and/or unsuitable for high-throughput organoid culture and analysis *in situ.* To facilitate dynamic organoid culture in a high-throughput screening (HTS) system, we have developed a pillar plate and a complementary perfusion well plate and "microarray 3D bioprinting" technology, which is highly flexible, user-friendly, and easily combined with conventional 384-well plates to support organotypic cell cultures and multiplexed high-content imaging assays. We have successfully demonstrated reproducible and scale-up production of human organoids on the pillar/perfusion plate for predictive compound screening. Several human organoids including brain, liver, and intestine have been printed, encapsulated in biomimetic hydrogels, differentiated, and imaged on the pillar/perfusion plate. Interestingly, dynamic culture of human organoids on the pillar/

perfusion plate significantly enhanced the maturity of human organoids potentially due to rapid diffusion of growth factors and small molecules necessary for cell differentiation and maturation. The optically clear pillar/perfusion plates allowed direct visualization of organoids on the pillars for predictive cell-based assays. The entire organoids on the pillar plate were permeabilized, fixed, stained with primary and secondary antibodies, and cleared with tissue clearing solutions simultaneously for *in situ* whole organoid imaging without the need for cryosectioning. The flexible pillar and perfusion well format connected by microchannels and reservoirs made it easy to change growth media for organoid culture without the use of bulky pumps and tubes. It is compatible with standard 384-well plates and existing HTS equipment including fluorescence cell imagers and microtiter well plate readers, which is an important feature for developing HTS assays. It is easy to connect different types of organoids cultured on the pillars by using the perfusion well plate, which is critical to simulate human diseases. Thus, the unique pillar/perfusion plate platform could offer new opportunities for creating highly organized organoids by dispensing human cells in hydrogels precisely with printing robots and mimicking the microenvironment of tissues *in vivo*, thereby potentially revolutionizing regenerative medicine, oncology, and drug discovery.

Presentation: Oral

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Modeling corticospinal tract pathophysiology with ALS iPSC-derived corticospinal motor neurons

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iPSC-derived cell subtypes are powerful tools for generating relevant human models of ALS pathophysiology. While differentiation protocols for spinal motor neurons (SPMNs) have been capable of producing relatively enriched populations of ChAT+ SPMNs, current cortical neuron differentiation protocols produce many neuronal and glial subtypes, of which corticospinal motor neurons (CSMNs) account for a relatively small proportion. COUP-TF interacting protein 2 (CTIP2) is required for the formation of the corticospinal tract (CST) and is the most commonly used marker for subcerebral projection neurons, including layer V CSMNs. Specifically enriching for, and identifying, CSMNs within iPSC-derived cortical cultures is particularly important for modeling ALS, given that ALS is characterized by specific degeneration of the CST, and that cortical dysfunction is an early pathological feature of the disease.

We are generating a fully human, iPSC-based model of the (CST) in ALS with a translationally relevant focus on layer V, CTIP2+ CSMNs. CTIP2+ CSMNs (hiPSC-CSMN), spinal motor neurons (hiPSC-SPMN), cortical astrocytes (hiPSC-cA), and spinal astrocytes (hiPSC-spA) are cultured in microfluidic devices for long-term compartmentalized co-culture, where the cortical cells are separated from spinal cells by microchannels. The combination of microfluidic devices with multielectrode array (MEA) plates allows for assessment of neuronal firing and functional connectivity of hiPSC-CSMNs and hiPSC-SPMNs in spatially separated cortical and spinal compartments. Following transduction of cortical neurons with Camkii-driven channelrhodopsin, optic stimulation of co-culture results in firing of both cortical and spinal compartments, suggesting that the two compartments are synaptically connected.

We hypothesize that ALS hiPSC-CSMNs will be hyperexcitable and contribute to impaired connectivity with hiPSC-SPMNs compared to healthy controls. Differentiation of CSMNs from ALS patients represents a precision medicine approach for testing drugs suitable for future clinical trials. Region specific hiPSC-A's will bolster the maturation of hiPSC-MNs, in addition to laying the groundwork for examining effects of astrocytes with specific ALS mutations related to CST pathobiology in ALS. Co-cultures between regionally-defined neurons and astrocytes provide a highly relevant and manipulatable model that will allow us to better understand ALS pathophysiology and test therapeutic strategies to impact CST health and connectivity.

Presentation: Poster

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Human induced pluripotent stem cell-derived hepatic organoids as an alternative *in vitro* model for toxicity testing

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The liver plays an important role in the detoxification and metabolism of drugs and compounds. Hepatotoxicity refers to impaired liver function in the process of metabolizing an administered drug, and is a major cause of drug withdrawal from the pharmaceutical market. A limitation of current *in vitro* models for hepatotoxicity testing is the loss of drug-metabolizing function due to the low expression and activity of cytochrome P450 (CYP450) enzymes. In

this study, we established a stepwise protocol to generate hepatic endodermal organoids from human induced pluripotent stem cells (hiPSCs) and subsequently differentiated functional hepatic organoids with high CYP450 gene expression and activity. Hepatic organoids had multicellular composition and exhibited major hepatic functions, including albumin secretion, glycogen storage, and drug transport. They also recapitulated the metabolic clearance and CYP450-mediated drug toxicity. Compared with primary human hepatocytes, hepatic organoids showed comparable or higher drug sensitivity to well-known DILI (drug-induced liver injury) drugs. They also reproduced the CYP450-mediated drug metabolic processes by detecting the metabolites of parent drugs such as acetaminophen and fimasartan. Therefore, these results suggest that hiPSC-derived hepatic organoids can be applied to CYP450-dependent drug toxicity testing as well as drug metabolism studies.

Funding sources: This research was supported by the Korea Institute of Toxicology (1711159818) and the Technology Innovation Program funded by the Ministry of Trade, Industry & Energy (20009350).

Presentation: Poster

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Pillar and perfusion plate enhanced cell growth, reproducibility, throughput, and user friendliness in dynamic 3D cell culture

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Three-dimensional (3D) cell culture has been demonstrated in ultralow attachment (ULA) well plates, hanging droplet plates, microtiter well plates with hydrogels or magnetic nanoparticles, and well inserts. These methods are simple, reproducible, and relatively inexpensive, thus potentially used for high-throughput screening (HTS) of compounds. However, statically cultured 3D cells often suffer from the necrotic core due to limited nutrients and oxygen diffusion and waste removal and have limited *in vivo*-like tissue structure. Here, we overcome these challenges by developing a high-throughput pillar/perfusion plate platform and demonstrating robust dynamic 3D cell culture for compound screening. As proof-of-concept, Hep3B cell spheroids generated in the ULA plate were transferred to the pillar plate with alginate by simple sandwiching and encapsulation and cultured in static and dynamic conditions to compare their cell growth and viability. Both spheroids cultured in the ULA plate in a static condition and on the pillar/perfusion plate in a dynamic condition showed similarity in size. The coefficient of variation obtained was below 20%, indicating high reproducibility of the spheroid transfer. Interestingly, there were many dead cells observed in the core of Hep3B spheroids cultured in the ULA plate due to diffusion limitation whereas dynamic spheroid culture on the pillar/perfusion plate significantly enhanced cell survival and growth by preventing cell death in the core. The viability of Hep3B spheroids in the ULA plate was 2-fold lower despite its large size. Cell death in the core was not because of oxygen depletion but because of nutrients depletion, which were determined by Image-iT green hypoxia assay and qPCR analysis of anti-apoptotic and pro-apoptotic markers. Unlike traditional dynamic cell culture in spinner flasks and microfluidic devices, the pillar/perfusion plate accelerated flow rates under the pillars in the perfusion wells, was compatible with common lab equipment, and allowed cell culture, testing, staining, and imaging *in situ*. Overall, the pillar/perfusion plate enhanced cell growth significantly by reducing the necrotic core, reproducibility by robust spheroid transfer, assay throughput by cell staining and imaging *in situ*, and user friendliness without using external pumps and tubes in dynamic 3D cell culture.

Presentation: Poster

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Integration platform for organoids and organ-on-a-chip by modularized technologies to control and sensing microenvironments with CUBE

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Organoid technologies that mimic the rich complexity of cell population and extracellular matrix (ECM) components of *in vivo* tissues contribute greatly to furthering our understanding of various biological phenomena. Nevertheless, it remains a challenge to exert control over complex shape and architecture, as well as tissue-tissue interactions of cultured organoids. The current organoid development is highly depending on cellular self-organization, but the homogeneous culture conditions *in vitro* cannot provide spatial information to cells correctly. Engineering principles, on the other hand, are great tools that enable us to tailor the design, composition, and construction of organoids according to the intended purpose of the study.

Here, we have developed *in vitro* experimental platform for the organoid culture to design and control microenvironment. The simple cube device, which comprises a polycarbonate frame with

rigid agarose walls and an inner ECM hydrogel, can be used to (i) control the spatial distribution of cells by employing 3D-printed carbohydrate moulds to create cell seeding pockets in the ECM hydrogel, (ii) design tissues with localized ECM by isolating ECM hydrogels of varying the composition, growth factors, or cells contained in separate compartments, (iii) facilitate integration with microfluidics for regulating the concentration gradient of morphogens to direct cell growth and differentiation, as well as connecting organoids for tissue-tissue interactions, and (iv) obtain 4D imaging by rotating the cube along with the diagonal axis to provide laser scanning from 3 orthogonal planes. By employing the platform, we have achieved to locally differentiate iPSC as well as to branch pattern formation with symmetry breakage at a desired location. The platform does not require complicated external system such as pump and laser. Thus, it can be easily installed in most of lab environment to accelerate the research for organoid developments. Organoids with desired morphology allows us to integrate them with artificial channels in a fluidic chip to link multiple organs.

Presentation: Poster

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Multiplexed superfusion system for physiological emulation: From concept to product

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The artificiality of the unphysiological cell culture conditions routinely used in biomedical research undermines the predictive value of cell studies. There has been continuous MPS development to emulate *in vivo* cellular microenvironments through culture media composition, chemical gradients, substrate biophysical and mechanical properties. Nonetheless, the highly dynamic nature of the native soluble microenvironment is frequently overlooked in *in vitro* systems. Evidently, fluidic (su)perfusion is a standard solution to this pervasive and critical deficiency. However, practical concerns over workflow integration hinder the routine fluidic implementation. In particular, there is a lack of any practical multiplexed active flow system that is economical, robust, and compatible with the existing cell culture practice, especially in larger-throughput industrial applications. We therefore fabricated a highly compact and multiplexed pump head (Rotary Planar Multiplexed Micropump, RPM2) offering 96 active pump lines in a small (7 x 7 x 12 cm) footprint to generate 0-60 µL/min flow rates. The pump is incorporated into an open-top superfusion system configured for conventional microplates or custom MPS interfaces. We have demonstrated the system utility in *in vitro* circadian maintenance through a daily hormonal zeitgeber (cortisol) pulse, generating a sustained circadian behaviour that is essential for physiological emulation and precision in chrono-pharmacological research, with further implications for all biomedical research. In addition, supported by our industrial partner, the multiplexed pump is now modularized and complemented by a comprehensive range of fluidic management methods enabling user-friendly and scalable operation.

With concurrent in-house biochemical sensor development and easy integration to standard/commercial MPS products, our suite of technologies provides a much-needed improvement in throughput and automation for physiological emulation, including the use of dynamic nutrient, hormone regulation and potentially pharmacokinetic simulation, to support the predictive value of *in vitro* biomedical research. Whilst earlier attempts to superfuse cell culture have mostly focused on specialised closed-channel designs with considerable limitations in practicality, our modular flow system is intended to transform the way *in vitro* biology is conducted by enabling high multiplexing capacity, open-top superfusion, and direct integration and compatibility with existing cell culture workflows.

Reference

[1] Gao, X., Wu, Y., Cheng, T. et al. (2022). *Lab Chip 22*, 1137-1148. doi:10.1039/D1LC00841B

Presentation: Poster

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HepG2 cells as a cell model for studying acute hepatotoxicity in the Emulate Organ Chip System

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The Emulate Organ Chip System is one of the few direct-to-consumer micro physiological systems currently available. However, the low number of protocols using primary human cells are a barrier to research groups adopting this technology. HepG2 cells provide an alternative to primary human hepatocytes (PHHs) when modelling non-alcoholic fatty liver disease and correlate with an r value of 0.81 with PHHs at a whole genome level. This study aimed to establish the viability of HepG2 cells under dynamic flow conditions in the Emulate system.

Emulate S-1 chips were activated, coated in a collagen/fibronectin mixture, and seeded with either 2 x 10^6 or 5 x 10^5 HepG2 cells/ml

in the top channel. Cells were overlaid with Matrigel and cultured under 30 µL/h flow for 72 h. At 24, 48, and 72 h images of the cultures were captured, and cell viability determined by urea, albumin, and lactate dehydrogenase levels in the spent media. Cholesterol and triglyceride production was also determined. At 72 h, chips were fixed, incubated with DAPI nuclear stain, and imaged using a Nikon ti2e microscope.

At 72 h, cells seeded at 2 x 10^6 cells/ml were overgrown with visible vacuole formation, whereas cells seeded at 5 x 10^5 cells/ml formed a confluent monolayer. In spent media, a transient increase in lactate dehydrogenase occurred at 24 h. At 2 x 10^6 cells/ml, albumin production increased by 72% and 145% at 48 and 72 h respectively, compared to 24 h. At 5 x 10^5 cells/ml, albumin production increased by 43% and 110% at 48 and 72 h, respectively, compared to 24 h. Cholesterol and triglycerides were not detected in the spent media. At 72 h, cells remained adherent at both seeding densities, confirmed by brightfield imaging and robust DAPI fluorescence.

This study indicates that for acute toxicity testing, HepG2 cells can be cultured under dynamic flow at 5 x 10^5 cells/ml in the top channel of Emulate S-1 chips. This model will be used to investigate the effects of fructose, fatty acids, and tamoxifen on non-alcoholic fatty liver disease.

Presentation: Poster

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The next generation lab-on-chip platform deploying real-time metabolic sensing

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Conventional drug discovery is a long and costly process, and it fails with much higher frequency than it succeeds. Amongst the many factors that play a role in these failures is the limited human pathophysiological relevance of the preclinical models used to qualify agents to progress to clinical phase evaluation. As an alternative to conventional models, lab-on-a-chip (LoC) has recently emerged as a new platform for preclinical drug research. To support this LoC paradigm real-time measurement of analyte concentrations in cell culture medium is critical to ensure high predictability and reproducibility in drug discovery. Moreover, compared with the hyper-nutritional composition of conventional medium (CM), dynamic supply of human plasma-like medium (HPLM) better emulates *in situ* tissue perfusion. An electrochemical aptamer-based (EAB) sensor for *in vivo* phenylalanine measurement in living rats has been established [1]. This EAB sensor has the potential for comparing LoC cell metabolism in different media in real-time. We aim to develop new EAB sensors that integrate pH and oxygen sensing with metabolite sensing (e.g., phenylalanine) into LoC, and, into dynamic cell culture perfusion setting to compare CM and HPLM using multiple tumor cell lines.

Based on the square wave voltammetry (SWV), the EAB sensor detected the concentration of phenylalanine in the physiologically relevant range between 30µM to 300µM. The data could be fitted to the Langmuir-Hill Equation with R-square greater than 0.99. The EAB sensor shows promising stability and repeatability between different electrodes and upon multiple independent manufactures.

Altogether, the sensor provides a useful method for *in situ* monitoring cell culture in real-time. Moreover, our study established the sensor's potential as a robust and reliable tool to support drug development and screening by validating the physiological status of the media. The sensor may be integrated with a pump-valve feedback control system to clamp the metabolic environment.

Reference

[1] Idili, A. et al. (2021). *Anal Chem 93*, 4023-4032.

Presentation: Oral

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Angiogenesis-on-chip: hiPSC-derived endothelial cell-line dependent angiogenic responses

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Angiogenesis, the development of blood vessels from preexisting vasculature, is key to the understanding of tissue repair, tumor growth, metastasis, inflammation, ischemic processes, endothelial dysfunction, microvasculopathies [1], and genetic vascular inherited disorders such as type 1 and 2 hereditary hemorrhagic telangiectasias (HHT1 and HHT2). HHT exhibits abnormal angiogenesis leading to organ-specific arteriovenous malformation development [2]. These complex disorders required advanced biological models to a) understand disease mechanisms b) recapitulate human physiopathology, and c) explore translational re-

search. 3D systems are suitable platforms to investigate angiogenic processes, which comprise cell signaling, endothelial cell specification, proliferation, migration and invasion. Moreover, the use of hiPSC-derived endothelial cells (hiPSC-ECs) represents an extra layer of complexity to the system, with the advantage of recapitulating the genetic background of the patient [3]. However, the reproducibility, robustness, and high-throughput of hiPSC-ECs-based angiogenesis-on-chip assays are currently limited for disease modeling and drug testing. We aimed to 1) provide insights on the use of multiple hiPSC-ECs control lines, and 2) establish an angiogenesis-on-a-chip protocol to study the functionality of HHT2 iPSC-ECs. Type I collagen (1,5 mg/ml) was injected into the gel channel of the AIM Biotech idenTx40 chips® followed by fibronectin coating of the upper medial channel, and EC seeding (4x106 cells/ml) for 24 hours. Angiogenic stimuli were applied in the bottom media channel to create a gradient and follow over 2 days with daily medium refreshments. Our preliminary results indicate that control lines respond differently to VEGF, DAPT, VEGF/DAPT, and VEGF/DAPT/S1P/bFGF. Also, sprouts are observed in a growth factor concentration-dependent manner. We hypothesize that the genetic background of the hiPSC-EC lines shapes the angiogenic response and might require additional hPSC line-to-line optimization. In conclusion, we have established conditions for sprouting angiogenesis-on-chip for several independent hiPSC-EC lines that could be next used to investigate HHT2-hiPSC-ECs.

Funded by the Netherlands Organisation for Health Research and Development (ZonMw) (PTO446002501), and the Novo Nordisk Foundation Center for Stem Cell Medicine grant (NNF21CC0073729).

References

[1] Eelen, G. et al. (2020). *Circ Res 127*, 310-329.
[2] Arthur, H. M. and Roman, B. L. (2022). *Front Med 9*, 973964.
[3] Vila, M. et al. (2021). *Stem Cell Rep 16*, 2159-2168.

Presentation: Poster

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Vascularization of multi-organ-on-chips with blood and lymphatic endothelial cells for the generation of immunocompetent skin models

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Background: Until now, 3D human skin models fail to include both blood and lymphatic endothelial cells despite their essential role for homeostasis and immune responses, limiting their relevance for disease modeling and safety testing. Blood endothelial cells (BECs) provide nutrients and mediate the entry of immune cells into inflamed tissue. Lymphatic endothelial cells (LECs) support tissue fluid homeostasis and support the transport of soluble antigens and immune cells to draining lymph nodes for immune surveillance and activation. To recapitulate these events, establishment of vascularized Organ-on-Chip microfluidic bioreactors with BECs and LECs are a pre-requisite to further improve human skin models to study human diseases.

Aim: Set up a robust and reproducible method for the vascularization of organ-on-chip microfluidics with human BECs or LECs which allows long term culturing under physiologic flow conditions for future immunocompetent (multi-) organ-on-chip models.

Methods: Endothelial cells from human skin were separated into BECs and LECs, expanded to generate pure populations, used to vascularize multi-organ-on-chip microfluidic bioreactors and cultured for up to 14 days under dynamic flow conditions mimicking blood and lymph flow pressures. Morphology, mRNA expression and biomarkers profiles in culture supernatants was investigated upon homeostatic and inflammatory conditions.

Results: The new method results in large numbers of highly pure BECs and LECs, which can be used to vascularize organ-on-chip devices. Upon prolonged culture periods, cells retained their endothelial specific phenotype. Mimicking blood vessel flow clearly induced morphological changes as the cells aligned in the direction of the flow, while this does not occur when applying lower lymphatic vessel flow. Biomarker expression of BECs and LECs was clearly different but not influenced by flow conditions. BECs and LECs respond to inflammatory conditions by upregulating soluble ICAM, VCAM, CCL2 and IL-6. mRNA levels of endothelial junction markers (Cldn5, VEcadh, ZO1) did not change in BECs and LECs, while the LEC specific marker Prox-1 was clearly reduced in LECs upon inflammatory conditions.

Conclusion: The presented method can be used to further enhance organ-on-chip models through the incorporation of functional BECs and LECs resulting in relevant healthy and diseased tissue models to investigate human disease and safety testing.

Presentation: Poster

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Vascularized hiPSC-derived 3D cardiac microtissue on chip

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Functional vasculature is essential for delivering nutrients, oxygen and cells to the heart and removing waste products. Current 3D organoid and microtissue models often integrate self-organized microvascular-like networks. However, these networks are often not functional and nutrient delivery in these models solely depend on the passive diffusion as they are maintained in static cultures without perfusion. Here, we developed an *in vitro* vascularized cardiac microtissue model based on human induced pluripotent stem cells (hiPSCs) in a microfluidic organ-on-chip by co-culturing hiPSC-derived, prevascularized, cardiac microtissues (MTs) with vascular cells within a fibrin hydrogel. We showed that vascular networks spontaneously formed in- and around these MTs and that they were lumenized and interconnected through the process called anastomosis. Anastomosis was fluid flow dependent whereby perfusion increased vessel density and thus enhanced formation of the hybrid vessels. Vascularized cardiac MTs showed altered contractile dynamics as a result of enhanced endothelial cell-cardiomyocyte communication and these contractile dynamics were reversed upon inhibition of endothelial cell-derived nitric oxide. Finally, stimulation with interleukin-1β induced production of proinflammatory cytokines and led to consequent changes in contractile parameters only in vascularized cardiac MTs but not in non-vascularized MTs in chips. These results highlight the importance of vascularization in inflammatory response. The platform sets the stage for studies on the role of organ-specific endothelial cell barrier upon delivery of drugs or inflammatory mediators.

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 812954; Netherlands Organ-on-Chip Initiative, an NWO Gravitation project funded by the Ministry of Education, Culture and Science of the government of the Netherlands (024.003.001) and the Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729).

Presentation: Poster

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Bioprinting in organ-on-chip for studying cancer metastasis in lymphatic vessels

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Bioprinting techniques have been increasingly applied in organ-on-chip (OoC) technology, to produce more complex structures, that will be able to replicate, more accurately, the processes that occur in living organisms [1]. Cell patterning, via bioprinting, materializes as a sterile and automatic mechanism of introducing cells in OoC platforms, while offering significant cell ratio control which in turn will provide high levels of biomimicry [1]. In this work, we investigate the metastatic behavior of cancer cells in lymphatic vessels by depositing and culturing cells in OoC platform, while perfusing the device with cell culture media. In particular, Laser-induced forward transfer (LIFT) is employed as a 3D bioprinting technique, to rapidly immobilize cells [2], in specifically designed chips, in order to imitate cancer tissue microenvironment. The design of the microfluidic chip includes culture chambers, as well as microchannels, so as to replicate blood and lymphatic flows. For chip fabrication, a DLP 3D printer is utilized in combination with a biocompatible, translucent resin. In order to optimally simulate the lymph fluid and blood plasma, appropriate cell culture media [3] are being diffused in selected channels of the chip. Microfluidic performance is validated by CFD simulations and flow visualization experiments.

The research work is supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 11421).

References

[1] Chliara, M. A., Elezoglou, S., and Zergioti, I. (2022). Bioprinting on organ-on-chip: Development and applications. *Biosensors 12*, 1135. doi:10.3390/bios12121135

[2] Karakaidos, P., Kryou, C., Simigdala, N. et al. (2022). Laser bioprinting of cells using UV and visible wavelengths: A

comparative DNA damage study. *Bioengineering 9*, 378. doi:10.3390/bioengineering9080378

[3] Ubellacker, J. M., Tasdogan, A., Ramesh, V. et al. (2020). Lymph protects metastasizing melanoma cells from ferroptosis. *Nature 585*, 113-118. doi:10.1038/s41586-020-2623-z

Presentation: Poster

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A quantitative modeling workflow for the design, analysis, and interpretation of experimental studies in gut-liver organ-on-a-chip systems

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Recent advances in Organ-on-a-chip (OoC) technology promise to enhance the predictability of *in vitro* assays for drug efficacy and safety. The greater physiological relevance of OoC systems better reproduces the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs compared to conventional *in vitro* systems. To fully leverage the great potential of OoC systems in preclinical studies, the development of complementary quantitative approaches is required to enable translation of the results to the *in vivo* situation and ultimately to human.

Quantitative mechanistic modeling of the PK/PD processes taking place in the multi-organ systems is an essential element in designing the OoC experiments, which will also enhance their translational value. Mathematical modeling is further required for the analysis of the data generated by the OoC systems to characterize temporal PK/PD relationships. Lastly, extrapolating PK/PD parameters to *in vivo* also depends on mathematical approaches.

Here, we present a MATLAB-based quantitative modeling tool to support all stages of experimental studies in OoC systems with combined gut and liver organ models. We have developed graphical user interface (GUI) applications as digital twins of different commercially available OoC systems.

Our quantitative modeling application is a user-friendly tool with capabilities that range from interactive experimental design (optimizing adjustable parameters to maximize similarity between the OoC systems and organs *in vivo*), to simulation (exploring the impact of parameters on the outputs of interest), data fitting (estimation of PK and toxicity parameters), and *in vitro* to *in vivo* extrapolation (IVIVE). Overall, our quantitative modeling tool provides a multi-purpose user-friendly GUI application with multiple functionalities for the design, analysis, and interpretation of experimental studies in gut-liver OoC systems.

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Enabling next generation functional characterization of human neural organoids

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Human organs, such as the brain, are challenging to study being inaccessible to direct optical observation and experimental manipulation. However, three-dimensional (3D) culture techniques enabled novel study of tissue- or stem cell-derived organoids, spheroids, and organotypic cultures resembling cell type diversity, developmental processes and function of mammalian organs.

The ability to measure the electrical activity of a self-organizing *in vitro* cellular model in real time, live and label-free can provide much needed insights into the complexity of its functional structure. High-density microelectrode arrays (HD-MEAs) provide unprecedented means for non-invasive *in vitro* electrophysiological recordings, and can be used to acquire real time measurements from any cell with electrogenic properties, such as iPSC-derived neurons, retina and brain organoids, as well as tissue slices.

In this study, a HD-MEA platform featuring 26,400 electrodes per well (MaxWell Biosystems AG, Switzerland) was used to capture fast propagating extracellular action potentials in neural organoids at different scales, ranging from network through single-neuron with high spatio-temporal resolution and low noise. Metrics, such as firing rate, spike amplitude, network burst profile as well as synchronicity, were extrapolated in a parallelized manner. Furthermore, at the subcellular level, we tracked the propagating action potentials across axonal branches to compute and characterize the conduction velocity across multiple neurons within a network.

Our HD-MEA platforms and the extracted parameters highlighted in this study provide a powerful user-friendly approach for identifying and isolating active areas of a 3D culture in acute recordings or in longitudinal studies allowing long-term disease modelling and/or compound testing *in vitro*.

This work is funded by the NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission. This work is funded by the HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.

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Towards improving maturation of 3D muscle-like constructs using cyclic mechanical strain in a pneumatically actuated platform

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Mechanical stimuli have been shown to play a critical role in the development of cell structure and functionality. Studies have shown that applying static and cyclic strains to mimic *in vivo* conditions can improve the *in vitro* cell maturity [1]. However, most of the current methods for applying mechanical stimulations are for 2D cultures, and still remain challenging for 3D cultures in aspects such as sample preparation and throughput. To address this issue, we developed an open-top, robust and easy-to-use system and used it to study the effects of mechanical stimulation on the maturation of muscle-like constructs.

The platform consists of three components: a pneumatically actuated chip, a pressure-based flow control unit, and the control system (Fluigent). The chip consists of two parts. The top is a 12-well culture plate made from stainless steel for 3D culture, and it can be magnetically attached to the bottom substrate without leakage. The bottom consists of a thin PDMS membrane bonded to a 1 mm thick fused silica slide, which has embedded micro-ducts made with a femtosecond-laser-assisted-etching process. The membrane and the slide are bonded using a double-sided silicone adhesive, which is pre-cut with CO_2 laser to match the shape of the wells.

Here we operate the device to provide a controlled and robust cyclic biaxial strain up to 30% at a frequency up to 1 Hz. To assess the effect of the system on cell viability, C2C12 myoblasts in collagen hydrogels were stained and analyzed for up to 7 days; fluorescent images indicated few cell death. Different straining regimes will be integrated to the tissue constructs during the differentiation process towards myotubes and an improvement in the muscle network formation is expected.

In conclusion, we developed an easy-to-use and high-throughput device to explore the effect of mechanical deformation on the development and maturation of 3D muscle-like cultures. For future application studies, we will use the platform in combination with cardiac microtissue to study tissue maturation over time in response to mechanical strain.

Reference

[1] Carlos-Oliveira et al. (2021). Current strategies of mechanical stimulation for maturation of cardiac microtissues. *Biophysical Reviews 13*, 717-727.

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Efficacy evaluation of AAV delivered liver specific promoters in the emulate liver chip

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Adeno-associated virus (AAV) derived vectors emerged as a promising gene delivery vehicle for a broad range of clinical indications. Among them, the liver is an interesting target as it is easily transduced with AAV vectors and allows a persistent expression of the transgene. To develop and optimize novel AAV vectors, advanced and complex *in vitro* liver models based on primary cells are required. The emerging "organ on-chip-technology", promises a better translatability from simple *in vitro* cell line models used for initial higher throughput screening, to animal models or human patients.

Here, we report a mini-screening of liver specific promoters performed on the Emulate liver chip using a transduction assay mediated by AAV vectors. Constructs with the mCherry reporter gene driven by five different enhancer-promoter control regions (4 candidates, 1 positive control) have been packaged into an AAV-DJ capsid, characterized by its broad tropism and efficient liver transduction. The AAVs were applied to both "channels" of the Emulate liver chip, seeded with primary human hepatocytes in the top channel, and primary human liver sinusoidal cells, stellate cells and Kupffer cells in the bottom channel and cultured under flow after transduction. The transduction efficiency in both channels was monitored by live cell imaging and endpoint quantification of viral genomic DNA and mCherry mRNA.

Results showed that in the positive control group, the mCherry fluorescent signal was readily detected from the second day after transduction in both channels, and increased until the end of the experimental timepoint. In contrast, comparing to the positive control, the tested liver specific promoters induced similar mCherry signals in the top channels, while being weaker or undetectable in the bottom channels. Molecular analysis confirmed these observations. Despite of similar viral genome copies detected in both channels, the liver specific promoters resulted in more mCherry transcripts in top channels and fewer in bottom ones.

Collectively, we show that our proprietary liver specific promoters are preferentially active in human hepatocytes, but not in non-parenchymal cells. Such organ-on-a-chip technology is useful for the efficacy evaluation of gene therapy candidates delivered by AAVs.

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Enhanced vascular organization in a vessel-on-chip model containing hiPSC-derived astrocytes

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The blood-brain-barrier (BBB) tightly regulates transport of molecules in and out of the central nervous system. Precisely regulated crosstalk between endothelial cells (ECs), pericytes and astrocytes is essential to the development and stability of the BBB. Disruption of this crosstalk is becoming a hallmark of many neurodegenerative disorders. There are currently few experimental models mimicking these complex interactions seen *in vivo*. Here, we developed a 3D vessel-on-chip (VoC) model which incorporates human induced pluripotent stem cell (hiPSC)-derived ECs (hiPSC-ECs), human brain vascular pericytes (HBVPs) and hiPSC-derived astrocytes (hiPSC-Astros). The vascular cells and astrocytes were combined within a fibrin hydrogel and self-organized into lumenized microvascular networks, with direct interaction between the cell-types included. hiPSC-Astros and human primary astrocytes (pAstro) behaved very similarly in VoC triple cultures with no apparent differences in the morphology or expression of contractile- and reactive markers in either HBVPs or astrocytes. We then sought ways to further improve vascular organization. We showed that continuous perfusion of the vasculature or activation of cAMP signaling significantly enhanced vascular organization in the 3D VoC-hiPSC-Astro model. This model is now well-suited to study brain specific vascular (patho)physiology.

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Engineering an Immunocompetent induced pluripotent stem cell-derived alveolus-on-chip to model infection

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Human alveoli are unique tissue microenvironments that play significant roles in respiratory diseases and pharmacological interventions. Local inflammatory responses serve as gatekeepers for multiple respiratory diseases and infections that are interfered by therapeutic molecules. To gain better insights into the alveolar functions and offer a scalable human pulmonary model for therapeutic assessment, we set out to establish an immunocompetent lung-on-chip model aiming to recapitulate the air-liquid interface and 3D mechanical stretching. Multiple efforts have attempted to recreate an alveolar micro-physiological system *in vitro*. Yet, these systems are crippled by the lack of microenvironmental features, donor-to-donor variation or heterogenous cell sources that lead to immuno-incompetency. To overcome such caveats, we harnessed the strength of induced pluripotent stem cell (iPSC) technology to derive the four key cell types of an immunocompetent alveolar microenvironment, including alveolar epithelial cells Type II and I, vascular endothelial cells, and macrophages from a single iPSC source. We have validated the differentiated cell identities by morphology, marker expression, cellular functions, and ultrastructural features. To recreate the 3D alveolar microenvironment, we assembled our iPSC-derived lung-on-chip (iLoC) on the state-of-the-art ^{AX}Lung-on-chip system [1] to achieve air-liquid interface and cyclical 3D mechanical stretching. Our iLoC system offers a rapid establishment of durable barrier function together with the prolonged retention of cellular markers of each cell type. Contributed by the accessibility design of the ^{AX}Lung-on-chip system, our iLoC system represents the first-reported single-cell source iLoC of four cell types compatible with various experimental interventions and analytical assays.

Reference

[1] Stucki, J. D. et al. (2018). Medium throughput breathing human primary cell alveolus-on-chip model. *Sci Rep 8*, 14359.

Presentation: Poster

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Human three-dimensional multicellular liver platform for drug screening

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The pharmaceutical industry is currently facing increasing challenges in drug discovery due to long drug development times combined with low clinical trial success rates. Over 20% of approved drugs suffer from market withdrawal for severe liver injury at therapeutic doses, becoming a significant cause of death worldwide. Three-dimensional (3D) cellular models, obtained by mixing cells with a biomaterial in a pre-specified structure, are emerging as a physiologically relevant cellular microenvironment holding a great promise for drug safety and efficacy in the early phase of drug development.

Here, we developed human 3D livers combining hepatocytes (HepaRG), hepatic stellate cells (HSCs) (LX-2), and immune cells (THP-1), the main cell types of the liver. We used a well-established mixture of gelatin methacryloyl (GelMA) and carboxymethyl cellulose methacrylate (CMCMA). Lithium Phenyl(2,4,6-trimethylbenzoyl)phosphonate was used as a photo-initiator. Cells were combined with the GelMA-CMCMA mixture and exposed to UV light for 30 seconds. The 3D livers were kept in culture for up to 30 days. They were challenged with acetaminophen and LPS, two well-described hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage *in vitro*.

The 3D livers did not show any signs of distress while resembling many characteristics of the native healthy liver. We evaluated the cell viability, morphology, and gene expression of hepatocytes, HSCs, and immune cells in the 3D livers during the 30 days. The LPS and acetaminophen challenge induced extensive liver damage characterized by HSC activation and proliferation. These cells acquired the typical myofibroblast phenotype of activated HSC *in vivo*. Moreover, we assessed gene expression markers of hepatocyte functionality, as well as the CYP3A4 enzyme activity, which were impaired upon treatments. The inflammation level of the system was measured by the transition from monocytes to macrophages releasing proinflammatory cytokines into the medium.

Human 3D livers are suitable for long-term cultures and can be used to test the liver toxicity of drugs, as has been demonstrated with LPS and acetaminophen. These results also validated this multicellular system as a valuable tool for measuring liver damage and inflammation, as the 3D livers successfully displayed the characteristics of liver damage *in vitro*.

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The application of advanced tools in next generation risk assessment (NGRA) of cosmetics ingredients

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Performing safety assessments for novel chemicals without using any animal data, particularly for complex endpoints such as systemic and DART is a significant challenge. Our strategy has been to create tiered and exposure-led frameworks for skin sensitisation, DART, systemic and inhalation toxicity, built on the principles developed by the International Cooperation for Cosmetics Safety. The aim of the first tier is to capture the potential bioactivity of any new chemical using high throughput new approach methodologies (NAMs) with broad biological coverage in a dose-response manner. Initial case studies with cosmetic ingredients (coumarin and phenoxyethanol) have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure (POD) derived from NAMs to make a safety decision for systemic toxicity [1,2]. This led us to systematically evaluate the toolbox of NAMs employed in the case studies (*in vitro* pharmacology profiling, cell stress panel and high-throughput transcriptomics in multiple 2D cell lines) for 10 different chemicals using 24 benchmark exposure scenarios [3]. These early tier tools showed promise for use in a protective rather than predictive capacity but demonstrated that the tier 1 might be overly conservative given that measures of chemical potency are based on bioactivity, which may not necessarily translate into adverse effects in humans3. Therefore, advanced organ models, including microphysiological systems (MPS) have the potential to be used as a refinement tool when a decision with a low tier approach could not be made. The potential areas of application of MPS in NGRA include both the use of individual organ systems (e.g., explore specific mechanisms of toxicity or transport mediated-toxicity) and multiorgan-on-a-chip to investigate kinetics, metabolism, and organ-to-organ communication (e.g., endocrine system). The rationale and selection of advanced tools for ongoing case studies, where the use of the systemic toolbox [3] was not sufficient to make a safety decision, will also be discussed. MPS are very promising, however fit-for-purpose evaluation in the context of use is essential to assure future confidence in the application of MPS in NGRA.

References

[1] Baltazar, M. T. (2020). *Toxicol Sci 176*, 236-252.
[2] OECD (2021): IATA for Phenoxyethanol.
[3] Middleton, A. M. (2022). *Toxicol Sci 189*, 124-147.

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A novel islet platform for studying type 1 diabetes and investigating β-cell proliferation

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Type 1 Diabetes is an autoimmune disease characterized by severe loss of β-cell function and mass. Current research models lack many functions critical for understanding the onset and progression of this disease in humans. Furthermore, restoration of β-cell mass by inducing proliferation of residual β-cells represents an attractive therapeutic approach for diabetes treatment. Here, we developed human islet microtissues (hIsMTs), which are derived from primary human islets, are homogeneous in size and composition, and have a culture span of 28 days. To enable studies of the immunopathogenesis of T1D, we established three islet-immune injury models by culturing islet microtissues with proinflammatory cytokines, activated peripheral blood mononuclear cells, or HLA-A2-restricted preproinsulin-specific cytotoxic T lymphocytes. In all three T1D models, β-cell function declined, as manifested by increased basal and decreased glucose-stimulated insulin secretion (GSIS) and decreased intracellular insulin content. Additionally, loss of the first-phase insulin response (FFIR), increased proinsulin-to-insulin ratios, HLA-class I expression, and inflammatory cytokine release were also observed. Using these models, we show that liraglutide, a glucagon-like peptide 1 receptor agonist, prevented loss of GSIS under T1D-relevant stress, by preserving the FFIR and decreasing immune cell infiltration and cytokine secretion.

In addition to using hIsMTs to perform T1D modeling, we also established a novel method for evaluating compound effects on β-cell proliferation and function. Following treatment with the proliferative agent harmine and EdU incorporation, islet MTs were stained in 3D for DAPI (nuclear marker), NKX6.1 (β-cell marker), and EdU (proliferation marker), imaged on a high-content confocal microscope, and subjected to automated 3D analysis of cell populations per islet MT. We observed by high-content confocal microscopy that 4 days of harmine treatment increased β- and non-β-cell proliferation, NKX6.1 expression, and basal and stimulated insulin secretion in a dose-dependent manner, whereas fold-stimulation of secretion peaked at intermediate doses. Our results highlight the value of hIsMT-immune injury models in the discovery and evaluation of novel compounds for the treatment of T1D. Furthermore, the additional methodology presented constitutes a unique tool for *in vitro* high-throughput evaluation of changes in human β-cell proliferation.

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Developing novel tools for diabetes research: AAV serotype tropism screen in standardized human islet microtissues

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Controlled modification of gene expression constitutes a promising therapeutic strategy for the treatment of numerous diseases, as well as an invaluable tool for dissecting their underlying pathophysiological mechanisms. However, targeted gene delivery to specific cell types remains challenging. The use of adeno-associated viruses (AAVs) for gene expression modulation represents a safe and effective viral gene delivery tool. Compared to other viral transduction systems, AAVs are advantageous due to their low toxicity to host cells as well as long-term transgene expression. Furthermore, different AAV serotypes differ in their tropism, which can be leveraged to preferentially transduce specific cell types. However, AAV serotype screening and therapeutic research are hindered by the lack of relevant human disease models. To address this need in diabetes research, we have established AAV transduction methodologies in highly standardized human islet microtissues. To achieve high transduction efficiencies in a 3D spheroid system, we perform optimized dissociation and controlled scaffold-free reaggregation of primary human islet cells in the presence of AAVs. This process allows for manipulation of gene expression in pancreatic endocrine cells at their single cell state, while enabling production of uniform, homogeneous islet microtissues that display long-term (> 28 days) and robust function.

In this study, we used this system to compare the tropism of 8 AAV serotypes encoding eGFP in primary human pancreatic islets. We used 3D confocal microscopy and established a semi-automated 3D image analysis to determine the count of successfully transduced eGFP-expressing cells. Furthermore, we assessed the effect of viral transduction on microtissue functionality and viability by measuring insulin secretory function, insulin content and ATP content. We found that the serotype AAV2.7m8 exhibited superior transduction efficiency in 2 different human islet donors,

while maintaining islet microtissue functionality and ATP levels. In summary, we present a novel diabetes research tool for the screening of AAV serotype tropism for pancreatic islets and a scalable system that may be used for efficient gene expression modification in functional, reaggregated islets by AAV transduction.

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Development of human B-cell lymphoma-on-chip to study cancer dissemination

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Diffuse large B-cell lymphoma (DLBCL), the most common and aggressive type of B-cell non-Hodgkin lymphoma, preferentially spreads to distant lymph nodes (LNs) upon dissemination, indicating that LNs provide the ideal tumor microenvironment. LN stromal cells (LNSCs), including lymphatic endothelial cells (LECs) and fibroblastic reticular cells (FRCs), form a scaffold for immune cell retention, activation, and migration [1], but their role for DLBCL dissemination is unclear. A lack of suitable *in vitro* and *in vivo* models resembling DLBCL makes it a challenge to investigate the complex lymphoma/LNSC interactions in the tumor microenvironment, and the effects of therapeutic interventions.

In 2D and 3D FRC/DLBCL co-cultures, we observe increased membrane expression of the cancer-associated fibroblast (CAF) markers [2] podoplanin and fibroblast activating protein (FAP), as well as vimentin compared to FRCs alone. These findings resemble FRC phenotype in tumor tissue from DLBCL patients, where we observe increased podoplanin and vimentin expression compared to non-tumor lymph nodes. Furthermore, FRCs in co-culture with DLBCL cell lines increase production of soluble factors important for tumor B-cell survival and migration – BAFF, CXCL12, CXCL13, CCL19 – indicating that LNSCs are adapting their functions with respect to support of tumor cell survival and migration. In support of this, 2-out-of-4 DLBCL cell lines migrated towards LNSCs in a transwell migration assay, suggesting that LNSCs play a role in tumor cell migration in specific DLBCL subtypes.

We are currently generating human B-cell lymphoma-on-chip as an innovative model to investigate dissemination of malignant B-cells. For this, we use a lumen-based microfluidic chip with three interconnected luminal lanes casted in PDMS [3]. In the middle channel, we culture FRCs in a collagen/fibrin hydrogel into which we observe migration of DLBCL tumor cells, which was again specific for some DLBCL subtypes. In next experiments, we will incorporate LECs to mimic a lymphatic vessel in the third lane thus completing the DLBCL dissemination model. We expect that our lymphoma-on-chip model will provide a platform for testing therapeutic drugs inhibiting lymphoma/LNSC interactions contributing to cancer cell dissemination.

References

[1] Grasso, C. et al. (2021). *Trends Immunol*.
[2] Davidson, S. et al. (2021). *Nat Rev Immunol 2021*.
[3] Jouybar, M. et al., unpublished

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Overcoming oxygen impermeability in PDMS-free organ-on-a-chip devices with nanoporous plastics

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We present a novel method to incorporate oxygen permeability in PDMS-free Organ-on-a-Chip (OoC) construction. Non-PDMS materials like thermosets or thermoplastics (used, e.g., via hot embossing, 3D printing, xerographic lamination, reaction injection molding, etc.) all suffer from low oxygen permeability. Under perfusion, this issue is negligible, as dissolved oxygen is supplied through the medium flow. But in static conditions, such as required immediately after seeding cells into an OoC to ensure attachment, hypoxic conditions might arise. Hypoxia can increase cell stress, influence stem cell fate, or even lead to cell death [1]. Companies like Ibidi or AIM biotech solve this issue by incorporating oxygen permeable membranes. However, currently available membranes are based on silicone/PDMS, fluoropolymers, or polyurethanes, which require relatively complex bonding in plastic device construction.

Here, we demonstrate how a track-etched commercial nanoporous membrane can provide oxygen permeability. Made from polycarbonate (PC; 25 µm thick, 1.2% porosity, 50 nm pores),

it is compatible with well-established plastic-plastic bonding processes, including our own OSTE+ and PC/tape OoC process flows [2,3]. With dry gas measurements (nitrogen to air), we establish a membrane permeability of 2780 ± 160 barrer (N = 2); ~5× higher than typical PDMS. Bonded to microfluidic channels from both 3M 9877 medical tape or OSTEmer 322, membranes remain leak-tight for 24 h at 333 mbar liquid pressure and burst-resistant up to 1bar (N = 3). Finally, we assemble single-channel OoC devices integrating Pyroscience OXSP5 oxygen sensors. Filled with PBS, we find that porous-membrane OoCs yield a pseudo-exponential equilibration time constant of 6.4 ± 3.4min when moved from air into a nitrogen-flooded incubator, over 50× faster than equivalent OoCs made with non-porous PC (N = 11). Conversely, in preliminary experiments (N = 4) with cultures of C2BBe1 colorectal adenocarcinoma or U87 glioblastoma (138 k or 100 k/cm^2 respectively), we observe that porous-membrane OoCs maintain constant 84 ± 2% oxygen saturation, compared with a marked ~16%/hour drop in the "regular" OoCs during the initial 1-2 h seeding phase.

In conclusion, nanoporous PC membranes facilitate simple and robust plastic-OoC process integration and rapid and reliable oxygen exchange.

References

[1] Palacio-Castañeda, V. et al. (2022). *Lab Chip 22*, 6.
[2] Matthiesen, I. et al. (2021). *Small 17*, 32.
[3] Winkler, T. E. et al. (2020). *Lab Chip 20*, 7.

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Butyrate improves host defense against *Candida albicans* infections in an inflammatory bowel disease on chip model

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Introduction: During inflammatory bowel disease (IBD), chronic inflammation of the host and microbial dysbiosis both negatively affect the gut barrier function. The short-chain fatty acid butyrate has been demonstrated to foster intestinal tissue self-renewal and to dampen detrimental inflammation by eliciting anti-inflammatory responses. There is emerging evidence that the yeast *Candida albicans* plays a crucial role in IBD pathogenesis, by eliciting detrimental inflammation [1]. Therefore, we hypothesize that butyrate may play a role in restraining opportunistic *C. albicans* infections by comprising positive effects on host tissue integrity.

Methods: We leveraged our previously established immunocompetent gut-on-chip model [2] to establish an IBD phenotype through luminal perfusion with 1.5% 40kDa dextran sodium sulfate (DSS). After 24 h of DSS treatment, the intestinal tissue was infected with *C. albicans* and 1 mM butyrate in the lumen was evaluated for its effect on fungal colonization, tissue invasion and translocation to the vascular compartment.

Results: DSS-induced inflammation caused increased fungal growth at the luminal side resulting in higher translocation through the gut barrier quantified by CFUs. A three-dimensional image-based analysis confirmed the formation of larger and denser *C. albicans* microcolonies in the IBD model under flow conditions. Interestingly, this effect was antagonized by butyrate, which induced a macrophage-dependent tissue self-renewal. Through stimulation of epithelial cell proliferation and improved junction complex formation, butyrate limited fungal colonization and tissue invasion.

Conclusion: Our findings support the beneficial role of butyrate as a microbial metabolite capable of preventing chronic intestinal inflammation and counteracting *C. albicans* infections in IBD patients.

References

[1] Li, X. V. et al. (2022). Immune regulation by fungal strain diversity in inflammatory bowel disease. *Nature 603*, 672-678.
[2] Maurer, M. et al. (2019). A three-dimensional immunocompetent intestine-on-chip model as in vitro platform for functional and microbial interaction studies. *Biomaterials 220*, 119396.

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Comparative *in vitro* DILI characterization of two candidate drugs using advanced *in vitro* liver models

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Inhibition of the aldo-keto reductase family 1 member C3 (AKR1C3) has been described as potentially beneficial for patients with endometriosis or PCOS. Our first selective AKR1C3 inhibitor BAY 1128688 (frontrunner, FR) underwent preclinical safety assessment according to ICH guidelines including 13 weeks repeat-dose toxicity studies in female rats and female cynomolgus monkeys: no indication of hepatotoxicity was observed at multiples of exposure of at least 16. *In vitro* transporter interaction studies demonstrated inhibitory activity of the FR for several hepatic uptake and efflux transporters also including the bile salt export pump (BSEP). A safety margin could still be calculated for the transporter inhibition IC50 values (1-10 µM) compared to the predicted therapeutic exposure. Since no other prohibitive preclinical safety findings had been observed, clinical development was progressed up to a 12-week phase IIa study in patients with endometriosis. In this study signs of liver injury were observed in several patients after treatment of more than 8 weeks (publication submitted). Root cause analysis including modeling and simulation (DILIsym simulation) collectively suggested inhibition of BSEP as one potential cause of DILI.

While aiming to identify novel AKR1C3 inhibitors without this liver safety liability, potential candidates were characterized, especially for absence of hepatocyte bile acid transporter inhibition. One follow-up candidate (FU) was identified which was not associated with signs of hepatoxicity during thorough preclinical *in vitro* and *in vivo* assessment. To further support clinical development of FU, both FR and FU were subjected to comparative *in vitro* characterization with respect to DILI risk in advanced *in vitro* liver models. We selected two models, the Emulate human liver chip and InSphero human liver microtissues (hLiMT), based on their ability to flag the FR liability and thus allow for identification of novel candidates devoid thereof. Relevant functional and viability parameters measured in these models suggest a clear difference between the two compounds, with FR showing a clearly increased risk for DILI which is not indicated for the FU candidate. These data will be discussed in detail.

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Laser assisted bioprinting of spheroids for the fabrication of organoids in microfluidic chips

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In recent years, 3D bioprinting has emerged as a suitable method for tissue engineering. Several printing techniques including ink-jet-based bioprinting, extrusion-based bioprinting, stereolithography and laser-assisted bioprinting (LAB) are under investigation. Among these techniques, LAB, which is based on the principle of laser-assisted forward transfer (LIFT), is the technique with the highest cell viability (> 95%) after the printing process [1], due to the fact that it is a nozzle/syringe-free printing technique, with less shear forces on the cells [2].

At the same time, it is often useful to build artificial tissues from already connected cell clusters such as spheroids, mimicking the extracellular matrix and enabling the establishment of vascularization [3]. As a proof of concept for spheroid transfer, spheroids made from 3T3 cells were produced in microforms of agarose and were investigated concerning accuracy, viability, and growth behavior of spheroids after the transfer. Finally merging of the spheroids and the ability to generate artificial tissue was investigated.

A laser at 2940 nm wavelength was used, to transfer the spheroid. The best results in resolution, spot size and shape were observed at a transfer distance of 1 mm. A pulse energy of 18 µJ at the sample resulted in a more successful transfer of spheroids compared to the use of 12 µJ.

Live/Dead staining showed that the viability of cells in the outer shell of the spheroids was reduced. Cells in the core were still alive after printing and grow as well as pipetted cells. This was verified with an MTT assay 2 h, 24 h, 48 h, and 72 h after the transfer. Using live-cell imaging for 24 h showed that cells spread from the printed spheroids and fill the spaces between those. An eight-day cultivation of printed spheroids resulted in a piece of inter-connected cells consisting of multiple layers.

The study demonstrates that LAB is useful for spheroid printing, to predesign larger tissues which can be used in Organ-on-Chip systems. Nevertheless, further investigations are necessary to improve cell survival and induce vessel formation.

References

[1] Barron (2005a). doi:10.1007/s10439-005-8971-x
[2] Murphy (2014). doi:10.1038/nbt.2958
[3] Walser (2013). doi:10.22203/eCM.v026a16

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Perfusable liver model for "on chip" disease modelling

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Background and aims: Liver related diseases such as non-alcoholic fatty liver disease, fibrosis, and alcoholic liver disease are global health problems with no specific treatments. A major limiting factor for developing new therapies is the lack of proper *in vitro* models to investigate the progression of the diseases. Our aim is to develop a perfusable liver lobule-on-chip system, which can be used for modelling of liver diseases and drug testing.

Method: In order to establish the method to build liver lobule "on chip" system, we designed a pumpless gravity-driven flow platform with a perfusable central vain surrounded by liver parenchymal cells encapsulated in extracellular matrix (ECM). HepG2, Human Umbilical Vein Endothelial Cells (HUVECs) and immortalized mesenchymal stem cells (MSCs) were mixed with 2 mg/mL type-1 collagen and Geltrex before loading into the ECM container of the chip following solidification. The perfusable channel was fabricated in the ECM mixture by removing a needle (560 µm) from the solidified cell-ECM mix. HUVECs were seeded into the perfusable channel after needle removal to line the walls of the channel. The channel was subsequently perfused using a two-dimensional tilting platform. After four days of culture with perfusion, the cellular constructs were fixed, and morphology was analyzed.

Results: The sections of the cellular constructs stained with HNF4-alpha, VE-cadherin, and DAPI showed that the cells self-organized and formed vascularized cellular constructs on the chip. The HUVECs encapsulated in the ECM formed a vasculature-like structure and the wall of perfused channel was lined with HUVECs. Results with primary- and stem cell-derived hepatocytes, stellate cells and endothelial cells are currently being carried out.

Conclusion: We present a scalable platform that enables combining a central perfusable endothelialized structure with surrounding parenchymal cells as a step towards a liver "sinusoidal-like" model. The platform can be useful for disease modelling and drug testing.

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Microfabrication of *in situ* functional neuronal networks using FluidFM for spheroid placement

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The use of spheroids and organoids in organ-on-a-chip technologies is gaining importance, particularly in neuroscience, as they can replicate features of the complex neural networks seen *in vivo* [1]. This comes with a demand to accurately position such microtissues within experimental microstructures to create well-defined, heterogeneous systems resembling physiological conditions.

Fluidic force microscopy (FluidFM) comprises an atomic force microscopy (AFM) cantilever, containing a hollow microfluidic channel [2]. Thus, the system merges the force-sensing abilities of AFM with the aspiration and dispensing abilities of a micropipette and can be used for the pick and place (P&P) of objects of interest.

Because of its gentleness, accuracy and optical access, FluidFM has been used extensively for the precise P&P of single cells [3], however has not yet been employed to P&P larger microtissues or spheroids.

The commercially available FluidFM OMNIUM is used to select specific neuronal spheroids from a cell-repellent substrate and place them in a polydimethylsiloxane (PDMS) structure. The cantilever is lowered until contact is made with the selected spheroid, detected using its AFM capabilities. A negative pressure is applied to the cantilever, and the spheroid is aspirated onto the cantilever's aperture. This can then transfer the spheroid to the location of interest, before depositing it here. The speed and accuracy of the system, as well as the viability and growth of the spheroids can be assessed.

A process flow is defined for P&P of spheroids using the FluidFM OMNIUM. This is then used to P&P spheroids of various sizes and construct functional neuronal networks, demonstrating the instrument's versatility.

This is useful in a variety of applications such as lab or organ-on-a-chip technologies where the precise placing of spheroids is required for experimental accuracy, with no adverse effects on the cells. Future work will develop a system for automated P&P of organoids, leveraging imaging recognition and automation.

References

[1] Vuille-dit-Bille, E. et al. (2022). *Lab Chip 22*, 4043-4066.
[2] Meister, A. et al. (2009). *Nano Lett 9*, 2501-2507.
[3] Martinez, V. et al. (2016). *Lab Chip 16*, 1663-1674.

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Micropumping chip module for a standardized and modular organ-on-chip platform

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Organ-on-Chip (OoC) is a game-changing technology in which human cells are cultured in microfluidic chips to mimic and predict the physiology and pathology of human tissues, as well as to provide insights into drug and disease mechanisms. However, current limitations in manufacturing and technical usability of existing OoC approaches must be overcome in order for industry and regulators to adopt OoCs. Our goal is therefore to develop a Standardized and Modular open-technology OoC platform as a new Approach to Recapitulate human Tissues (SMART OoC), that enables the integration of a novel microfluidic pump. This platform is a further development of the Translational OoC Platform (TOP), which includes a plate with an integrated microfluidic channel network with standardized fluid input/output ports, to which exchangeable chip modules can be connected [1]. To provide fluid pumping to the platform, we are developing a novel micropumping chip module based on magnetic artificial cilia (MAC) [2]. MAC are flexible rod-shaped magnetic micro-actuators inspired by biological cilia, made of polydimethylsiloxane (PDMS) containing magnetic microparticles. MAC can induce microfluidic flow and particle transport when integrated into a microfluidic module and actuated with an external magnet. In this research, we present a novel miniaturized actuation setup for actuating biocompatible MAC integrated in a versatile microfluidic module that generates a variety of fluid flow regimes in the OoC platform. In comparison to other micropumping methods, this module does not require tubings or electrical connections, which opens up a wide range of possibilities for OoC applications.

References

[1] Dekker, S., Buesink, W., Blom, M. et al. (2018). Standardized and modular microfluidic platform for fast Lab on Chip system development. *Sens Actuators B Chem 272*, 468-478.

[2] Toonder, J. M. J. den, Onck, P. R. (2013). Microfluidic manipulation with artificial/bioinspired cilia. *Trends Biotechnol 31*, 85-91.

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Establishing a quality management plan for microphysiological systems (MPS): Quality parameters and monitoring reproducibility

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In recent years, the field of cell cultures has undergone significant development with the goal of obtaining more relevant human data. Many researchers have moved away from classical single-cell and flat models to more complex models, such as organoids, which better mimic human tissue. The development of organotypic cultures, on-a-chip technologies, and other 3D cultures, also known as microphysiological systems (MPS), have shown great potential to generate human models that can reproduce human pathology more accurately. These new *in vitro* models enable advances in both basic research and biomedical applications.

However, due to the complexity of the protocols and the use of new bioengineering techniques in cell cultures, it is more challenging to standardize procedures to ensure reproducibility. There have been numerous efforts to establish adequate guidelines and quality controls for cellular models over the years. For example, Good Cell Culture Practice (GCCP) has produced an initial guideline in 2005, which has been updated by several manuscripts on MPS and with a new revised version of the GCCP guideline (GCCP 2.0). All this aligns with the internationally recognized OECD guidance document on Good *in vitro* Method Practice (GIVIMP) which is intended to support method developers and end-users working to establish new *in vitro* assay methods in academic, industry or government laboratories. Additionally, the Recommended Guidelines for Developing, Qualifying, and Implementing Complex *In vitro* Models (CIVMs) for Drug Discovery have been recently published.

We have now generated or produced a document that aims to provide guidance in the development of appropriate quality con-

trols for MPS to improve reproducibility and promote good cell culture practices on these new models. The document does not intend to impose detailed procedures but to describe potential quality issues and possible quality controls.

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NeuroExaminer 2.0 – microfluidics made entirely of glass for monitoring zebrafish brain activity using light-sheet imaging

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Zebrafish larvae are a vertebrate model system for neuroscience research. Their body are optically translucent and enable non-invasive observation of neural circuits throughout the entire brain [1]. However, to obtain accurate imaging of zebrafish, it is crucial to restrict its movements. Microfluidic technology improved neuroscience studies on Zebrafish regarding to brain-wide activity mapping. By positioning and immobilization, microfluidics allows to place Zebrafish in desired orientation and apply water soluble chemical stimuli with high spatiotemporal precision as already shown with a preliminary version of a NeuroExaminer [2]. Light-sheet microscopy, on the other hand, offers a unique possibility to investigate the entire brain in real time at single cell resolution and to gain novel insights into its function. Nonetheless, it has rarely been used in combination with microfluidic devices, as it requires imaging geometry that can only be realised with advanced microfabrication [3]. Easy to fabricate PDMS microdevices are widely used but have disadvantages that do not appear compatible with a commercial light sheet microscope. To overcome these drawbacks, we present an all-glass microfluidic system fabricated with femtosecond laser ablation that is compatible with fast whole-brain *in vivo* imaging with cellular resolution. NeuroExaminer 2.0 – entirely constructed out of glass microfluidics offers an approach for observing brain activity in response to chemical stimuli application in the sub second time range, while at the same time supplying larvae with a continuous flow of oxygenated media. NeuroExaminer 2.0 with relevant system design for undistorted coupling of light-sheet from two opposing directions into the brain and high-quality imaging from a third direction. With gentle fluidic manipulation and agarose-free immobilization, changes in brain activity of zebrafish can be long-term monitored and may thus provide novel insights into neuropsychiatric disorders.

References

[1] Ahrens, M. B., Orger, M. B., Robson, D. N. et al. (2013). Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods*.

[2] Mattern, K., von Trotha, J. W. and Erfle, P. (2020). NeuroExaminer: An all-glass microfluidic device for whole-brain in vivo imaging in zebrafish. *Commun Biol*.

[3] Albert-Smet, I., Marcos-Vidal, A., Vaquero, J. J. et al. (2019). Applications of light-sheetmicroscopy in microdevices. *Front Neuroanat*.

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Utilizing bioprinting technology to develop a 3D *in vitro* liver model

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The field of bioprinting has great potential for developing in-house, customizable organ models that would contribute greatly to aid in *in vitro* predictive toxicology efforts. Bioprinting technology utilizes both traditional additive manufacturing techniques with the relevant living cells to create physiologically relevant structures. This project used an Allevi1 bioprinter to print a combination of HepG2 cells and Pluronic F-127, which served as the bioink for the three-dimensional *in vitro* model. To ensure liver cell viability and functionality after printing, a live-dead assay and human albumin ELISA assay were utilized. Confocal microscopy was used to confirm the bioprinted liver cells were viable metabolically and structurally after printing. This project was the first bioprinting effort at the U.S. Army DEVCOM Chemical Biological Center and yielded successful results to serve as a reproducible *in vitro* model for future toxicity screenings.

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3D-gut-on-chip infection model of *Vibrio cholerae*

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Background: Cholera, a disease associated with acute and secretory diarrhea in humans, is still prevalent in many parts of the world. The disease is caused by *Vibrio cholerae*, a Gram-negative pathogen that colonizes the small intestine and harnesses the intestinal mucus as a nutrient source. During infection, *V. cholerae* secretes the cholera toxin, causing severe diarrhea as a result of increased chloride efflux. Since humans are the only naturally occurring mammalian host of *V. cholerae*, no adequate animal model is available. Thus, our work targets the lack of relevant models for this disease.

Methods and results: In our study, a human 3D-gut-chip [1] was infected with fluorescently labelled *V. cholerae* and cultivated under constant perfusion. The bacterial burden was investigated by CFU counts, immunofluorescence imaging and flow cytometry, revealing a stable colonization of the gut model. Even after several hours of infection, the epithelial and vascular tissue was fully intact, as proven by staining for ZO-1, E-cadherin, and VE-cadherin. Consequently, no bacterial translocation to the vascular side was detected. Besides the infection experiments, the impact of cholera toxin on the intestinal cells was analyzed. Repeated treatment of the gut chip with cholera toxin resulted in a higher cell count of mucin-2-producing cells. Preliminary results showed an increased signal of the chloride channel CFTR after cholera toxin treatment of the gut model. To back up this finding, the chloride efflux of the intestinal cells is currently investigated using chloride-sensitive fluorescent dyes.

Conclusion and outlook: We were able to cultivate *V. cholerae* for several hours in a microfluidic human 3D-gut-chip model while maintaining full tissue integrity and functionality. We were able to follow bacterial colonization and the impact of cholera toxin on CFTR and the chloride efflux. In future studies, the infection model will be used to study the modulation of the host response to colonization, to determine the role of mucus-derived secondary messengers in the context of *V. cholerae* quorum sensing, and to test novel treatment strategies for cholera disease.

Reference

[1] Maurer, M. et al. (2019). A three-dimensional immunocompetent intestine-on-chip model as in vitro platform for functional and microbial interaction studies. *Biomaterials*.

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Development and adaptation of a SARS-CoV-2 infection model in human respiratory MPS at high containment

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Since its emergence in 2019, SARS-CoV-2 has resulted in an estimated 680 million infections and almost 7 million reported deaths as of January 2023. The global response to the pandemic led to significant worldwide disruption to healthcare systems and economies. Periodic emergence of novel variants continues to slow the global recovery as these variants appear to be able to evade existing convalescent and vaccine elicited immunity and therapeutics. This has highlighted the need for physiologically relevant *in vitro* systems to assist pre-clinical research and development of novel therapeutics to help reduce our dependence on animal experimentation.

Microphysiological systems (MPS) provide an ideal platform for this type of infectious disease model development. We are adapting the Emulate organ chip system as a platform to model viral infection of human respiratory epithelial cells at high containment (Biosafety level 3, BSL3). Containing the equipment within a custom-built flexible film isolator (FFI) will provide a convenient way to safely work with SARS-CoV-2 at BSL3. This approach could also be taken to work with other pathogens at BSL3 and BSL4. Here we describe the engineering controls we have developed to perform these experiments and will present the characterisation of SARS-CoV-2 infection in a human respiratory MPS using authentic virus.

At UKHSA we have isolated all of the SARS-CoV-2 variants of concern throughout the pandemic and will assess these in future MPS experiments at BSL3. We will utilise a focus forming assay as a measure of viral replication in both the apical and basolateral compartments and will immunostain for presence of viral nucleocapsid in human respiratory epithelial and endothelial cells. The use of a Meso Scale Discovery V-plex assay will also permit the analysis of cytokine secretion into the basal media, a more physiologically relevant readout than transcript analysis alone. These proof of principle experiments will demonstrate the feasibility of using MPS at high containment and will pave the way for many further preclinical research and development studies.

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Comparative assessment of hepatic *in vitro* systems for detection of drug-induced liver injury

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Drug-induced liver injury (DILI) is a major concern for the pharmaceutical industry and a leading cause of preclinical and clinical drug terminations. This is due, among other reasons, to the diversity of mechanisms that can lead to DILI and to species differences. Cross-species liver *in vitro* models that closely resemble liver physiology are being developed and commercialized by many different vendors. The landscape of developers of such models is a fast evolving one and a fit-for-purpose approach is needed by the end users to understand which models bring the most value in the drug discovery pipeline. Work in our laboratory has investigated the potential of different liver co-culture models in 2D and 3D, with and without perfusion, to detect DILI. We will present the results of our fit-for-purpose comparative analysis as well as considerations around ease of use, robustness, reproducibility, and cost of the different systems tested.

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A multi-organ-on-a-chip device to study the metabolic crosstalk between muscle and pancreatic islets

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Diabetes mellitus is a chronic disease that represents a major public health problem worldwide. Type 2 diabetes (T2D) is the most common form of this disease, accounting for 90-95% of cases of diabetes and is characterized by hyperinsulinemia and insulin resistance. T2D is a complex metabolic disorder that comprises several organs. The pancreas is the organ with a critical role in T2D since in the pancreatic islets, beta-cells produce, store and release insulin. Skeletal muscle is one of the major tissues targeted by insulin and is responsible for maintaining whole-body glucose homeostasis. Understanding the crosstalk mechanisms between skeletal muscle and pancreatic islets is fundamental to developing new molecular drugs for this disease. In this work, we engineer a new *in vitro* multi-Organ-on-a-Chip (OOC) model to study skeletal muscle and pancreas. To this aim, muscle tissues and pancreatic islets have been fabricated and combined in a multi-OOC approach. Moreover, the multi-OOC device was integrated with a Localized Surface Plasmon Resonance (LSPR) sensing module to monitor the insulin and interleukin-6 (IL-6) secretion online and label-free. Using this *in vitro* platform, we have monitored insulin secretion dynamics by the pancreatic islets in response to the skeletal muscle contraction induced by electric pulse stimulation. These results point that this multi-OOC is an important enabling step for diabetes modeling, the study of insulin resistance, and the investigation of drug candidates for therapy, usually performed by long-time and expensive animal experiments. It would open new areas of research on human diabetes disease.

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Tunable hydrogel scaffolds to support 3D neuronal networks

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Biomaterial scaffolds have emerged as a tool to build 3D cultures of cells which better resemble biological systems, while advancements in bioelectronics have enabled the modulation of cell proliferation, differentiation, and migration [1]. Here, we first describe a porous conductive hydrogel with the same mechanical modulus and viscoelasticity as neural tissue [2]. Electrical conductivity is achieved by incorporating low amounts (< 0.3% weight) of carbon nanomaterials in an alginate hydrogel matrix, and then freeze-drying to introduce a highly porous network. The mechanical and electrical properties of the material can be carefully tuned and used to modulate the growth and differentiation of neural progenitor cells (NPCs). In addition to forming neurite networks that span the material in 3D, the NPCs can differentiate into astrocytes and oligodendrocytes. With increasing hydrogel viscoelasticity and conductivity, we observe the formation of denser neurite networks and a higher degree of myelination. Application of exogenous electrical stimulation can then be applied to the scaffolds to further promote NPC differentiation. To investigate the functionality of neurite networks in 3D, we begin by placing a polydimethylsiloxane (PDMS) microstructure on an underlying multielectrode array (MEA), as previously described [3]. We then explore different materials and techniques to integrate hydrogels into the PDMS microstructures, such that the hydrogel can facilitate neurons to form 3D networks while still confined by the PDMS. This platform is compatible with various methods to assess neuronal functionality (e.g., MEA electrical recordings), and can be used to understand the effect(s) of hydrogel properties on the resulting neuronal networks. Both described biomaterial platforms can support the growth of neuronal cells for over 6 weeks and could facilitate the development of biohybrid electronic devices to understand neuronal development and disease.

References

[1] Saydé, T. et al. (2021). Biomaterials for three-dimensional cell culture: From applications in oncology to nanotechnology. *Nanomaterials*.

[2] Tringides, C. M. et al. (2022). Tunable conductive hydrogel scaffolds for neural cell differentiation. *Adv Healthc Mater*.

[3] Forró, C. et al. (2018). Modular microstructure design to build neuronal networks of defined functional connectivity. *Biosens Bioelectron*.

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A novel integrated approach for proximal tubule-on-a-chip development

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The incidence of chronic kidney disease (CKD) is on the rise, highlighting the need for *in vitro* platforms that assist in understanding the mechanisms of kidney disease and developing new treatments. During the progression of CKD, the epithelial barrier of the proximal tubule (PT) severely deteriorates. 3-dimensional (3D) microfluidic technologies for PT disease modelling can provide new insights into the development of more precise therapeutic treatments. Our PT-on-a-chip model simultaneously incorporates the effects of topography and fluidic shear stress, using a stepwise integrated approach. This approach leads from design, selection, and modeling to device manufacturing, allowing for the optimization of device configurations and settings for optimal cell culture.

Two-channel chips were designed in AutoCAD with varying parameters (wave amplitude, frequency, and porosity). The porous interface was designed as a collection of cylinders whose arrangement allow for a controlled distribution of porosity. Optimization was performed *in silico* with computational fluid dynamics (CFD) in COMSOL, where the theoretical transport of molecules across the porous interface and shear stress profiles were simulated. The designs featuring the most suitable sheer stress and flow patterns were then transferred to small chips via soft lithography. Solute transport profiles across the device surface were systematically modeled using small fluorescent particles (20 μm). The *in silico*-microfluidic tandem model is the ideal platform from which to perform cell culture in the microfluidic device.

PT cells were successfully cultured in the device to form a monolayer with the *in silico* culture conditions. The modeling of the fluorescent particles in the device successfully indicated cell transport during cell seeding. This allowed culturing in the device whilst saving time and resources in trial-and-error cell seeding. Perfusion of the device during cell culture can generate flows that could impact the transport capacity of the cells [2].

The novel tandem approach in this study aided in the device development and establishment of a cell barrier in the microfluidic device, bringing us closer to developing a PT-on-a-chip and ultimately advancing the efficiency and discovery of kidney-on-a-chip models.

References

[1] Jansen, J. et al. (2015). *Sci Rep 5*, 16702.

[2] Masereeuw, R. et al. (2014). *Semin Nephrol 34*, 191-208.

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DMD-on-a-chip: Joining a functional patient-derived 3D skeletal muscle model, microfluidics and nanoplasmonic sensing to accelerate drug testing for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most prevalent neuromuscular disease diagnosed in childhood. It is characterized by a progressive degeneration of skeletal and cardiac muscles, caused by the lack of dystrophin protein. The absence of this structural protein leads to the fragility of the sarcolemma, and muscle fibers are damaged during their contraction. To date, there is no cure available for patients, even though there are several molecules in drug development. However, due to the limitations of preclinical research, the success rate of drugs remains low. In this work, intending to accelerate drug development for DMD, we developed an innovative organ-on-a-chip (OOC) platform to faster evaluate anti-DMD treatment candidates. This OOC consists of a microfluidic device that sustains the culture and electrical stimulation of up to six patient-derived 3D functional skeletal muscle tissues. Moreover, it is connected to a plasmonic sensing device that allows the monitorization of myotube integrity, closely related with anti-DMD drug effectiveness. The DMD *in vitro* model is developed by the encapsulation of myogenic precursors in a fibrin-composite matrix using a PDMS casting mold. Two flexible T-shaped pillars provides continuous tension to the tissue, allowing the orientation of the muscle fibers. DMD tissues, after continuous contractile regimes, reproduce the loss of myotube integrity that is observed in DMD due to the sarcolemmal instability. This can be monitored not only using functional phenotypes based on tissue contractibility, but also measuring Creatine Kinase (CK), a marker of muscle damage increased in blood-levels of DMD patients. Because of its relevance, we developed a nanoplasmonic biosensor for CK detection to fast, direct, label-free, and real-time monitor of sarcolemmal damage. Taking advantage of surface plasmon resonance phenomena, nanoplasmonic sensors are optical sensors that can detect biomarkers in the order of picograms. Altogether, the applicability of the DMD-on-a-chip in evaluating therapeutic compounds was explored. By testing potential up-regulators of utrophin, a dystrophin surrogate, we could select a promising drug candidate for the treatment of DMD. Taking all these considerations, this work shows that OOCs have great potential to be especially valuable in the development of drugs to treat DMD and other neuromuscular disorders.

Presentation: Poster

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Vascularized pancreatic islet-on-chip for type 1 diabetes

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Diabetes is a growing threat to global health, currently affecting 537 million people worldwide, or 1 in 10 adults. The World Health Organization predicts that 783 million people will suffer from diabetes in 2045. Diabetes, type 1 or 2, cause an abnormal and prolonged increase of the blood glucose level, mainly caused by deficiency or failure to use insulin. In the long term, diabetes can lead to many complications, including vasculopathy, neuropathy, retinopathy, and diabetic foot ulcer. There is a need to understand the mechanisms of action in a human model in order to finely-tune the treatment for each patient. Building *in vitro* analytical tools is crucial to model the complexity of physiology and pathophysiology of pancreatic islets for a better understanding of its basic biology as well as for the screening of new drugs. While most common perifusion systems require pooling of multiple islets to achieve quantifiable insulin concentrations, minimizing the number of islets required for experiments using microfluidic platforms is important given the scarcity of these biological tissues.

We have previously developed a microfluidic device integrating a network of hyper-elastic valves for automated glucose stimulation and insulin collection from a single pancreatic islet (Quintard et al., 2022). Here, we used the same serpentine chip design and cultured human beta cells spheroids composed of EndoCBH5 cells (from Human Cell Design), in a fibrin gel enriched in endothelial cells and fibroblasts. Thus, we tested the effect of flux by using a syringe pump in aspiration, and of an enriched vascular microenvironment on the functionality of the human beta cells spheroids. A glucose stimulated insulin secretion assay was performed and we observed an improved functionality of the human beta cells spheroids with both the flux and the enriched microenvironment. Our results confirm that recreating a favorable pancreatic islet niche improves functionality.

Our results pave the way to personalized medicine for the study of diabetes. The perspectives offered by this islet-on-chip model are considerable and promise to be beneficial to patients.

Presentation: Poster

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Exploring extravasation dynamics of lung cancer cells using a microvasculature on chip system

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Introduction: Lung cancer is associated with the highest mortality rate among all cancers, mainly due to late diagnosis at an advanced stage. To improve the prognosis of lung cancer patients, it is important to gain a better understanding of the precise mechanisms of the metastatic process, such as the extravasation dynamics of cancer cells to a distant site. Human 3D microvasculature-on-chip systems appear to be a suitable tool to study this poorly understood step during metastasis. Non-small cell lung cancer (NSCLC) accounts for nearly 80% of all lung cancers, which is why the A549 adenocarcinoma cell line has been established as commonly used lung cancer cell model. The aim of this study was to investigate the extravasation dynamics of two A549 subpopulations, holoclones and paraclones, on a microvasculature-on-chip system.

Material and methods: Primary human umbilical vein endothelial cells and normal human lung fibroblasts were co-cultured in a microfluidic platform. The cells were embedded in a fibrin hydrogel and self-assembled towards a perfusable and interconnected microvascular network within seven days. Further, A549 holoclones and paraclones were introduced into the mature network and incubated for 24 hours to investigate their extravasation potential and thus their metastatic capacity.

Results and discussion: In this study, holoclones and paraclones of the A549 adenocarcinoma cell line were shown to differ not only in their tumour initiation ability, toxicological sensitivity, and antioxidant profile, as previously reported [1], but also in their metastatic potential. Holoclones remained within the microvascular network, whereas paraclones extravasated into the surrounding hydrogel within 24 hours of incubation.

Reference

[1] Tièche, C. C., Gao, Y., Bührer, E. D. et al. (2019). Tumor initiation capacity and therapy resistance are differential features of EMT-related subpopulations in the NSCLC cell line A549. *Neoplasia (New York, N.Y.) 21*, 185-196. doi:10.1016/j.neo.2018.09.008

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A breathing lung-on-chip array incorporating a protein-based membrane

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Organ-on-Chip models of the distal lung rely on the fabrication of thin, porous, and, simultaneously, elastic cell culture membranes suitable for the co-culture of, for instance, lung epithelial and endothelial cells. The porosity allows direct cell-cell communication and culture at an air-liquid interface, while the elasticity allows for the integration of cyclic stretching of the cells, mimicking the breathing motion. However, improvements in the cell culture membrane should maintain the fabrication simplicity and usability of the chip.

The improved version of the second-generation Lung-on-Chip model [1] integrates a thin, collagen-based membrane in an injection-molded chip out of a Cyclic Olefin Copolymer (COC). The design in a microscope-slide format increases user-friendliness and compatibility with standard mounts in instrumentation which supports the ongoing and essential trend toward standardization in the field of Organs-on-Chip. The chip can support long-term cell culture for up to 30 days without bubble formation or leakage and is compatible with state-of-the-art readouts like transepithelial electrical resistance (TEER), PCR, and ELISA measurements. A numerical simulation of the strain pattern on the membrane was performed to define the amplitude of the cyclic stretching of the cell during the "breathing motion".

Continuous improvements of the membrane, which is embedded in a reliable, low-cost chip, will allow the study of more complex biological effects in lung diseases, such as emphysema, lung cancer, and lung fibrosis. Preliminary results of the recapitulation of the air-blood barrier will be presented.

Reference

[1] Zamprogno, P. et al. (2021). Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. *Commun Biol*.

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Incorporating primary human epithelial cells on a novel alveoli-on-chip device with diverse strain distribution

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Alveolar cells are set in an ever-dynamic microenvironment. The alveolar epithelium is continuously alternating between stretching and relaxing. Since a decade, organ-on-chips have enabled researchers to culture cells under similar dynamic and tissue-relevant conditions [1]. However, few of these mimic the strain distribution and structure of the alveoli [2]. To investigate the cellular effects of stretch, a novel alveoli-on-chip with *in-vivo* like dimensions and divers strain distributions was designed. The new consists of a polydimethylsiloxane (PDMS) membrane, whose stretch can be finetuned to define the resulting strain. Thereby, it can be cyclically stretched within the physiological and pathophysiological range. For a realistic and relevant cell culture, primary alveolar epithelial cells type 2 (phAEC2) are used. Those cells resemble the alveolar epithelial progenitor cell type. Adapting recent progress to maintain their differential cell state [3], we established a long-term culturing method of these cells within Matrigel, for on-demand dissociation. Once the Matrigel is dissociated, the phAEC2 can be seeded on the novel alveoli-on-chip. Together, the novel alveoli-on-chip and the possibility to harvest phAEC2 on-demand, will extend the understanding of cellular signaling, within the human alveolar epithelium. In our study, the novel alveoli-on-chip allows for the investigation on the effects of physiological and pathophysiological stretch on various cellular processes.

References

[1] Huh, D., Matthews, B. D., Mammoto, A. et al. (2010). Reconstituting organ-level lung functions on a chip. *Science 328*, 1662-1668. doi:10.1126/science.1188302

[2] Zamprogno, P. et al. (2021). Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. *Commun Biol 4*, 168. doi:10.1038/s42003-021-01695-0

[3] Youk, J. et al. (2020). Three-dimensional human alveolar stem cell culture models reveal infection response to SARS-CoV-2. *Cell Stem Cell 27*, 905-919.e10. doi:10.1016/j.stem.2020.10.004

Presentation: Poster

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Modeling a disease phenotype associated with preterm birth *in vitro* using a feto-maternal interface (FMi) organ-on-chip (OOC)

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Background: Infection and oxidative stress (sterile) associated inflammation during human pregnancy are two major risk factors for preterm birth (PTB), which is the leading cause of neonatal mortality and morbidity. Inflammation-associated cellular dysfunctions can create a disease state at the fetal membrane-decidual interface (feto-maternal Interface [FMi]) that leads to PTB. Unfortunately, it is impractical to study this disease state during human pregnancy, and thus difficult to mitigate and reduce the adverse pregnancy outcomes.

Methods: To address these limitations, an FMi organ-on-chip (FMi-OOC) platform was developed composed of four cell culture chambers containing immortalized primary fetal (amnion epithelial, mesenchymal, and chorion trophoblast) and maternal (decidua) cell that are interconnected by arrays of microchannels. Two different disease states of pregnancy (infection and oxidative stress) were recreated with this platform, with two distinct bi-directional pathways (ascending: maternal → fetal & intra-amniotic: fetal → maternal). Infection was modeled using lipopolysaccharide (LPS, 100 ng/mL) exposure, and oxidative stress was induced using cigarette smoke extract (CSE) exposure. The impacts of LPS and CSE were assessed by measuring cell viability (LDH assay), expression of the toll-like receptor [TLR] 4, disruption of immune homeostasis (immunostaining for HLA-DR and HLA-G), epithelial-to-mesenchymal transition (EMT) (cell shape index and immunostaining for cytokeratin-18/vimentin ratios), development of senescence (senescence-associated b-Gal staining), and inflammation (cytokine multiplex).

Results: Bi-directional LPS and CSE propagation was seen irrespective of treatment side over 72 hours and induced no cytotoxicity. However, distinct pathological changes were identified in both maternal and fetal cells. Fetal LPS and CSE exposure induced enhanced cellular pathologies and inflammation compared to treatments on the maternal side. However, maternal LPS and CSE treatment-induced immune intolerance within chorion or decidua cells.

Conclusion: Here, an FMi-OOC platform was used to model multiple disease states (infectious and sterile inflammation-associated PTB) of the FMi during pregnancy. The pronounced impact produced by the fetus supports the hypothesis that fetal inflammatory response is a mechanistic trigger for parturition. The FMi disease-associated changes identified in the FMi-OOC suggest the unique capability of this *in vitro* model in testing in utero conditions.

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Perfusable vascularized stroma on-a-chip for growing 3D organotypic structures

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Organoids and spheroids are recognized as key tools for *in vitro* studies, by partially recapitulating the structure/function of human tissues/organs. However, there are still barriers to unlock their full potential as 3D-models, such as limited oxygen/nutrients diffusion, often followed by growth arrest and metabolic waste accumulation, which may develop necrotic cores. While this may be relevant to represent specific developmental/disease stages, it also precludes tissue growth, maturation, and functional organization. To tackle this, we designed a microfluidic device recreating a perfusable vascularized stromal niche to support spheroid and/or organoid cultures.

The device had a central open reservoir with a inserted needle, which was filled with fibrin with i) no cells, ii) spheroids (with fibroblasts and EC-endothelial cells [1], and/or iii) intestinal organoids. The needle was removed after crosslinking creating a hollow channel, which was seeded with EC under optimized conditions. Perfusion was probed with fluorescent dextran and microbeads. Cellular organization was analysed by immunofluorescence/confocal imaging.

The cell-seeding density greatly impacted cell behaviour in the channel. At medium-to-high densities, EC assembled into a monolayer from which lumenized sprouts radially formed, invading the hydrogel, but this was abrogated at low cell-seeding density. Both the channel and sprouts could be efficiently perfused with no leakage. When spheroids were added to the system, they led to substantial outward cell migration and capillary-like structure formation. These were able to anastomose with the main channel, producing a perfusable vascular network. The device supported the growth and maturation of intestinal organoids in close contact with the vascularized stroma. Notably, the whole system could be recovered from the chamber without integrity loss, opening-up a wide-range of downstream analysis options.

Our device may combine parenchymal/stromal compartments with a perfusable vascular network. It can be adapted to the use of different spheroids and/or organoids, providing a unique *in vitro* platform for building biological-complexity and advancing the use of organotypic structures as 3D-models of human tissues/organs.

Acknowledgments: FCT-Portuguese Foundation for Science and Technology: project-PTDC/BTM-ORG/5154/2020, fellowship-SFRH/BD/2020.07458. REMODEL (857491-H2020-WIDESPREAD-03-2018).

Reference

[1] *Biomaterials 279*, 121222 (2021).

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Validation of 3D human liver-on-chip model as standard assay for ADME and toxicity predictions during pre-clinical development

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During preclinical development, safety-related issues often terminate development of drug candidates. Recently, it was estimated that only 10% of drugs entering Phase I clinical trials would eventually become FDA-approved therapies with drug-induced liver injury remaining a leading cause for attrition [1]. This high attrition rate is concerning as costs to bring these therapies to the market have increased significantly in the past decade. Although animal models are routinely used to predict safety and pharmacokinetic profiles, they lack translatability to human subjects. Therefore, more complex *in vitro* models using human cells have been developed as an additional tool for preclinical testing. These include three-dimensional (3D) spheroids and multicellular organoids which can accurately recapitulate organ-level functions *in vitro*. These models can be further incorporated into microphysiological systems (MPS) thus improving biochemical and biophysical cues which further improve the predictive power of the system. Nevertheless, a detailed characterization is needed to qualify MPS as a standard screening tool for use in the pre-clinical pharmaceutical drug development [2]. In this study, we aim to define the context-of-use and qualify 3D cell-based models, including commercially available 96- and 384-well plates, microwell plates and the MPS Humimic TissUse Chip 2. Furthermore, we compare the influence of media composition on the metabolic activity of cells cultured in these 3D platforms. To that extend, we used single-donor primary hepatocytes and probe the enzymatic activity of cytochrome P450 enzymes of three different donors. We demonstrate that regardless of the platform used, 3D liver spheroids exhibit a higher metabolic activity when cultured in William's E media when compared to other commercially available formulations. We further validate the platforms by screening a library of reference

compounds, demonstrating its potential to predict safety-related issues before entering the animal testing phase.

References

[1] Mullard, A. (2016). Parsing clinical success rates. *Nat Rev Drug Discov 15*, 447. doi:10.1038/nrd.2016.136

[2] Baudy, A. R. et al. (2020). Liver microphysiological systems development guidelines for safety risk assessment in the pharmaceutical industry. *Lab Chip 20*, 215-225. doi:10.1039/c9lc00768g

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Quantitative fluid dynamic characterization of an organ-on-chip model using phase resolved Doppler OCT

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Organ-on-chip (OoC) systems are novel microfluidic microsystems that combine the advantages of well-characterised human cells with the benefits of engineered, physiological-like microenvironments manufactured in the system. The extracellular matrix (ECM) is the natural microenvironment of cells in the human body responsible for providing the appropriate stimuli to cells to control cell processes such as proliferation, migration, and apoptosis. OoCs can mimic the ECM, via channels and porous membranes, by providing the cells with physiological-like mechanical stimuli governed by the fluid dynamics in the system [1]. Understanding the fluid dynamics in OOC can aid in fine-tuning the stimuli sensed by the cultured cells, understanding cell behavior and cell fate. The current state of the art methods for characterizing fluid dynamics in the OoC systems are simulations, theoretical calculations, and empirical observations, therefore a quantitative characterization technique is lacking. Optical coherence tomography (OCT) has been used in previous studies to measure omnidirectional flow velocities in flow systems [2].

In this study, we measured the flow in a cuvette using a Thorlabs GANYMEDE II HR series (high axial resolution of 3 mm in air) spectral domain OCT system. We made quantitative 2D flow measurements using the phase-resolved Doppler method. This work was then extended to extract flow dynamics, in the Bi/ond inCHIPit using titania scattering nanoparticles, which would be a novel way of flow characterization in the field of OOC. The results are compared to the theoretical Hagen-Poiseuille equations and COMSOL simulations and found to be in good agreement. The results of the study were further extended to determine the shear stress experienced by the cells in the culture well of the OoC.

References

[1] Menéndez, A. B. C., Du, Z., van den Bosch, T. P. P. et al. (2022). Creating a kidney organoid-vasculature interaction model using a novel organ-on-chip system. *Sci Rep 12*, 1-11. doi:10.1038/s41598-022-24945-5

[2] Cheishvili, K. and Kalkman, J. (2022). Scanning dynamic light scattering optical coherence tomography for measurement of high omnidirectional flow velocities. *Optics Express 30*, 23382. doi:10.1364/OE.456139

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Automated and high-volume wafer-scale microfabrication of organ-on-chip (OoC) polymer structures and components

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Organ-on-chip (OoC) technology is a promising improvement within *in vitro* cell culture, better mimicking functional units of human organs compared to conventional techniques. Current fabrication of three-Dimensional (3D) components in OoC, such as thin membranes and microfluidic structures, is often achieved via soft lithography, bonding, and punching of access holes of polymers, such as polymethylsiloxane (PDMS). However, these methods often suffer from the need of manual fabrication steps, drastically increasing production time and reducing yield due to handling errors and manual alignment of the layers. Consequently, the scalability is limited, which is a crucial aspect for a more widespread adaptation of OoC technology. In this work, we present a reproducible and scalable process for the direct patterning of various 3D polymer structures. The investigated process employs commercially available systems from IC packaging to mould pillars, membranes, and microfluidic channels with varying dimensions and thicknesses. Our process simultaneously improves the control over the thickness and dimensions of these structures in comparison to conventional fabrication techniques. Furthermore, proof of functionality is presented by adapting this technology to an existing OoC platform which incorporates integrated electrodes used for electrophysiological recording, stimulation, and TEER measurements. We demonstrate a complete process for wafer-scale microfabrication of OoCs, enabling low-cost, high-volume automated production. This is an important next step to large-scale manufacturing of

OoCs, enabling more biologists and scientists to integrate OoCs into their workflow.

References
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3D chip model to study cellular interplay in cancer cell invasion through Notch signaling

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The mechanoregulated Notch pathway controls cell fate decisions through juxtacrine signaling between neighboring cells and paracrine signaling via environmental cues. In the tumor microenvironment (TME), the role of Notch varies from tumor suppressor to oncogene, depending on the cancer cell type. Notch activation in the TME is influenced by factors such as extracellular matrix (ECM), hypoxia, inflammatory cytokines, and binding of ligands [1]. The complexity of TME makes it difficult to recapitulate the physiological and pathological aspects of the disease in a classical 2D cell culture. In contrast, 3D models may include molecular, chemical, and biomechanical components of the TME facilitating the study of tumor progression, invasion, and immune evasion in a relevant environment [2]. Current organ-on-chip models are great research tools to co-culture up to 4 cell types (cancer cells, cancer associated fibroblasts, endothelial cells, and immune cells) in 3D, and to incorporate fluid dynamics to simulate from blood flow to interstitial flow, among other aspects [3].

In this research, we benefit from the one-ligand-one-receptor fidelity of Notch signaling to study the cellular crosstalk in the TME. We use our in-house, easy-to-fabricate platform to investigate the role of the Notch ligand Jagged1 in highly metastatic triple negative breast cancer cells. Our device is made of PDMS bound to high-resolution imaging compatible glass, which allows *in vitro* observation of the cancer invasion in real-time. The design includes nine sets of two well compartments connected by a channel of 1 mm x 4 mm x 2 mm (LxWxH). The formation of an ECM filled channel allows the isolation and collection of cells and media from different compartments for biochemical analyses. Through our device, we aim to reveal the mechanistic insights of tumor and TME interactions, highly relevant for the advancement of future drug development and treatment.

References
[1] Meurette, O. and Mehlen, P. (2018). Notch signaling in the tumor microenvironment. *Cancer Cell 34*, 536-548.
[2] Mehta, P. et al. (2022). Microfluidics meets 3D cancer cell migration. *Trends Cancer*.
[3] Haessler, U. et al. (2012). Migration dynamics of breast cancer cells in a tunable 3D interstitial flow chamber. *Integr Biol 4*, 401-409.

Presentation: Poster

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Unified organoid system for modeling heart and kidney interaction on-a-chip

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Heart and kidney diseases cause high morbidity and mortality. Both organs have vital functions in the human body and reciprocally influence each other's behavior: pathological changes in one can damage the other. There are already multiple independent *in vitro* (human) models of heart and kidney, but none have so far captured their dynamic crosstalk [1]. Our aim is to develop a microfluidic system which can be used to study heart and kidney interaction *in vitro*. The validation of a unified organoid system will enable the investigation of diseases involving the two organs and their potential treatments.

The commercially available Ibidi µ-Slide III 3D perfusion chip was used for developing the combined culture of heart and kidney tissue on-a-chip. Cardiac microtissues (cMTs) [2] and kidney organoids (kOs) [3] derived from human induced pluripotent stem cells (hiPSCs) were loaded after 21 days from their formation in static culture onto two separated communicating chambers. We applied a unidirectional flow with a rate of 100 µl/min and the dynamic culture conditions were maintained for 72 hours. Tissue viability in the system was monitored and assessed by the beating of cMTs and the quality/presence of sarcomeres and nephron structures in cMTs and kOs, respectively. The tissues were then collected for downstream analyses. Functional characterization was performed through MuscleMotion to evaluate the contraction properties of cMTs and the uptake of albumin in kOs.

We expect this system will enable us to study the cardiac and kidney interaction with a high level of control and, where unidi-

rectional flow is used, to investigate how the heart affects the kidney or *vice versa*. This "cardio-renal-unit" represents a novel *in vitro* model for the study of the cardiorenal axis and its optimization will lead the way towards disease modeling on-a-chip.

References
[1] Gabbin B et al. (2022). *Front Cardiovasc Med.*
[2] Campostrini G, Meraviglia V et al. (2021). *Nat Protoc*.
[3] van den Berg CW et al. (2018). *Stem Cell Reports*.

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Engineered human cardiac chambers recapitulating the pump function of the heart

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Engineered 3D cardiac tissues have emerged as tools to model cardiac disease and accelerate the drug discovery pipeline. However, most models are subjected to static loads, limiting their ability to mimic the physiologic stress and strain experienced during cardiac cycles. Instead, newly developed engineered cardiac chambers have been explored due to their ability to recapitulate the pump dynamics of the heart, whilst providing access to clinically-relevant pressure-volume readouts. Here, we engineered cardiac chambers using a novel sacrificial-moulding approach and evaluated their pump performance in a non-invasive manner.

Two gelatin bodies were casted and aligned inside a 3D-printed bioreactor to create an ellipsoid shell cavity around a glass capillary. The gap established between the gelatin bodies was filled with a fibrin mix loaded with human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and human cardiac fibroblasts. Following fibrin polymerization, thermal degradation of gelatin was induced to obtain a single-inlet pumping cardiac chamber. Ejection volumes were quantified based on the displacement of liquid in the glass column during contraction of the chamber.

Our engineered cardiac chambers displayed spontaneous beating and pumping of fluid since day 7 post-fabrication. The tissues exhibited organized sarcomere structures and followed electrical pacing up to 2.5 Hz. The functionality of our model was further validated by positive and negative chronotropic responses to isoproterenol and nifedipine, respectively. Moreover, we performed live ultrasound imaging to analyse the dimensions of the chamber wall during beating cycles. Finally, we demonstrate the compatibility of our model with sustained cyclic mechanical stretching, highlighting its potential applications as a maturation approach for hPSC-CMs, or to introduce the modelling of cardiomyopathies.

Further biomechanical characterization and the incorporation of dynamic loads to the cardiac chamber will result in a more comprehensive model of the human ventricle. We envision that our model, in combination with the non-invasive acquisition of functional readouts, will bring research with engineered cardiac chambers to a higher throughput level.

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Organ-on-a-chip: Technology for the interface between the brain and the blood-brain barrier

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Organ-on-a-chip is an advanced technology that can mimic organ functions *in vivo* through 3D multicellular structures. It can enable accurate drug / toxic screening and help to understand the disease mechanism better. The blood-brain barrier (BBB) present in cerebrovascular vessels is a significant bottleneck in developing effective therapies for nervous system diseases, and therefore, a better understanding of the mechanisms of the formation of this barrier is of great importance in the development of therapeutics. However, it is not possible to accurately perform compound screening because there has not yet been an *in vitro* system that mimics this barrier structure and function. Here we show a BBB-on-a-chip system consisting of multiple neurovascular chambers that establish realistic BBB structures on a single chip. The effect of endothelial-astrocyte cell coculture and shear stress on barrier permeability was characterized by permeability analysis. Besides, drug tests, as well as neuro-inflammatory tests, were demonstrated on the BBB chip. The results showed that the chip closely resembles the brain and endothelial environment *in vivo*. In this regard, the chip can be used to accurately predict the BBB permeability of drug candidates in the preclinical stage, while at the same time being expected to be an alternative to minimize animal use.

Presentation: Poster

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Corneal toxicity screening: Successful replacement of rabbits by human *in vitro* corneal tissues

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The development of new drugs for treatment of ocular diseases via application to the front of the eye requires ocular toxicity testing to evaluate the potential hazardous side effects. Such tests are mainly performed by topical application of the test substance to the animal's eyes and evaluating the biological response. In order to reduce animal experiments and potentially increase human relevance, we developed an *in vitro* approach for corneal tolerability testing of drugs using an ALI (air-liquid interphase) human corneal epithelium (HCE). We refined an OECD-validated eye irritation test using a similar model by adapting the assay and implementing a repeated dosing approach to mimic the *in vivo* repeated dose setting and drug exposure as closely as possible. Furthermore, we added a fluorescent corneal surface damage staining as clinically relevant endpoint for a safety readout together with MTT, LDH, TEER and imaging-based readouts. We could demonstrate good translatability of *in vivo* ocular toxicity findings to our *in vitro* model by back translation of safety findings derived from a previous rabbit *in vivo* study. Hence, the *in vitro* corneal repeated dose assay became an early decision-making step in the screening cascade of ocular drug candidates. As a next step, we are working on integrating additional cell types in the safety assessment to more closely mimic the complex microenvironment at the front of the eye and thereby improve this *in vitro* assay as a screening tool.

Presentation: Poster

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Micropathological chip modeling the neurovascular unit response to inflammatory bone condition

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Organ-on-a-chip platforms accurately mimic complex microenvironments offering the ability to recapitulate and dissect mechanisms of physiological and pathological settings, revealing their major importance to develop new therapeutics. Bone diseases, namely osteoarthritis, are extremely complex, comprising of the action of inflammatory mediators leading to unbalanced bone homeostasis and de-regulation of sensory innervation and angiogenesis [1]. Although there are models to mimic bone vascularization or innervation, *in vitro* platforms merging the complexity of bone, vasculature, innervation, and inflammation are missing [2]. Therefore, in this study a microfluidic-based neuro-vascularized bone chip (NVB chip) is proposed to 1) model the mechanistic interactions between innervation and angiogenesis in the inflammatory bone niche, and 2) explore, as a screening tool, novel strategies targeting inflammatory diseases, using a nano-based drug delivery system.

We designed, optimized, and validated an advanced NVBchip model to address the intricate relationship between sensory neurons, endothelial cells, and osteoclasts, in bone physiological and inflammatory pathological conditions. For the first time, a microfluidic-based model was presented comprising fully differentiated mouse primary osteoclasts. Moreover, this is the first bone microenvironment model, including, not only the important vasculature contribution, but also the sensory innervation intimately connected to the pain perception.

The functionality of the NVBchip model was demonstrated by characterizing each cell type compartment by the evaluation of cell-specific morphology and cellular markers. Bone unit was successfully established with the presence of differentiated multinucleated osteoclasts, sustaining a high pro-inflammatory response and pro-angiogenic profile when exposed to IL-1β. The vascular unit was effectively achieved displaying the 3D tubular-like structure expressing CD31, while permeability studies confirmed the integrity of the endothelial barrier. The functionality of the neuronal unit was accomplished by the axonal growth response to the inflammatory stimuli. This system was validated by delivering anti-inflammatory drug-loaded nanoparticles to counteract the neuronal growth associated with pain perception. This reliable *in vitro* tool will allow understanding the bone neurovascular system, enlightening novel mechanisms behind the inflammatory bone diseases, bone destruction, and pain opening new avenues for new therapies discovery.

Funding: European Union's Horizon2020 research and innovation programme, grant No953121 (FLAMIN-GO – From pathobiology to synovia on chip: driving rheumatoid arthritis to the precision medicine goal).

References

[1] Tuckermann, J., Adams, R.H. (2021). *Nat Rev Rheumatol 17*, 608.

[2] Park, D. Y., Lee, J., Chung, J. J. et al. (2020). *Trends Biotechnol 38*, 99.

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No improvement in 60 years: Drug failure rates from the 1960s to the 2010s

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Drug efficacy, safety, bioavailability, and other pivotal drug properties are routinely assessed in animals throughout preclinical trials. Internationally, the evaluation of multiple nonclinical drug parameters in living animals is either required by law or regarded as best practice and encouraged by the responsible regulatory agencies like the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA). Simultaneously, the attrition rates during clinical drug assessment have been notoriously high, raising the question of whether current animal-intensive practices are adequate to test drugs for human use.

Here, we aimed to shed light on the development of new drug candidates' clinical failure rates in the last six decades. For this purpose, we analyzed the average attrition rates of drug candidates entering clinical Phase I from the 1960s to the end of 2010s. We investigated data reported in 19 studies for 52 partially overlapping periods and show that in the last 20 years the average drug failure rate is approximately 92%, comparable with the levels in the 1960s. Next, we analyzed the reasons for drug failure in one or several phases of clinical trials from 12 reports. Our results reveal that biological reasons such as safety and efficacy issues, classically assessed in animal trials throughout nonclinical drug evaluation, constitute approximately 76% of causes for drug attrition. Taken together, our analysis provides a detailed overview of the drug failure trends in the last 60 years. The fact that drug failure has remained at its highest point in the past 20 years despite the overall scientific and technological advances strongly suggests that the current animal-based preclinical assessment systems cannot be used as reliable predictors of human biology. Our findings indicate that more predictive, human-relevant systems are urgently needed in order to improve preclinical predictability and success rates in drug development.

Presentation: Poster

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Modeling of obesity-induced changes in metabolism and crosstalk of human stem cell-derived pancreatic islets and liver organoids using a pump-less recirculation OoC (rOoC) platform

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The worldwide pandemic of obesity is associated with a substantial increase in the prevalence of metabolic-associated fatty liver disease and type 2 diabetes mellitus. Both conditions are metabolically interlinked and often coexist in patients, calling for reliable human *in vitro* models mimicking the interactions between pancreatic islets and the liver.

We recently developed a novel scalable pump-less recirculation OoC platform (rOoC) that generates a directional, gravity-driven flow and allows to link islets and liver organoids "on chip" without the need of external tubing [1]. The dual rOoC iteration used in this study includes a membrane between two recirculation loops allowing directed transport of proteins from the islets to the liver compartment, while supporting the exchange of glucose and other small molecules between the circuits. It is therefore well suited to model and interrogate the inter-organ(oid) crosstalk.

The dual rOoC platform supported the long-term culture (for at least 2 weeks) of islet and liver organoids, evaluated by ATP content, concentrations of insulin and glucagon, albumin, and urea. The co-culture of pluripotent stem cell (PSC)-derived islet and liver organoids in the dual-rOoC was beneficial for the functionality of both tissue representations, when compared to static and on-chip monocultures. An improved glucose-stimulated insulin secretion (GSIS) and insulin/glucose consumption by liver organoids demonstrated a successful crosstalk between islets and liver organoids in the dual-rOoC. Supplementation of culture medium with free fatty acids and fructose, mimicking an unhealthy diet, caused significant changes in the morphology, metabolism, and functionality of both tissue representation alongside with increased levels of cytokines release, including IL6, CCL2, IL8, IL22.

In summary, we present a new scalable and easy-to-use pump-less OoC platform for the functional co-culture of islet and liver

organoids, enabling the crosstalk between them, and suitable for disease modeling and drug discovery studies.

Reference

[1] Busek, M., Aizenshtadt, A. et al. (2022). *Lab Chip*. doi:10.1039/D2LC00919F

Presentation: Poster

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Establishing a patient-derived glioblastoma organoids model that mimics tumor heterogeneity in patients

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Glioblastoma is the most frequent and malignant primary brain tumor. It carries a poor prognosis despite advances in surgery, radio- and chemotherapy. Human organoids are an excellent alternative to recapitulate a human organ-specific architecture and microenvironment *in vitro*. In this project, we propose an *ex vivo* glioblastoma organoid platform that is fully patient-derived, with tissue taken from different tumor sub-regions to account for intra-tumor heterogeneity.

To establish this model, we used patient navigated biopsies from three different sub-regions of glioblastoma (hypoxic/necrotic core, contrast-enhancing, and peritumoral infiltration zone). The procedure was based on an established protocol by Jacob et al. [1] The patient material was provided by the *Kantonsspital St. Gallen* (KSSG) in Switzerland.

The organoids were kept in culture for up to 6 weeks, during which two specimens were taken at different time points, and processed for immunohistochemistry (H&E, GFAP, MAP2, CD45, CD3, CD68, and Ki-67). The evaluation of the staining was done with the assistance of a neuropathologist. The growth of the organoids was quantified using brightfield microscopy pictures. A quality control was implemented based on the morphology and organoid-like appearance of the specimens.

The growth rate and quality of the organoids were patient-dependent. From two patients, we generated high-quality and fast-growing organoids. However, from the others we obtained low-quality organoids or nothing (from a recurrent case). There was no obvious morphological difference between the organoids from the three sub-regions.

The histopathology analysis showed that the organoids preserved features from the original tumor tissue: abundant pleomorphic nuclei (like high-grade gliomas) and the presence of macrophages, leukocytes (including T lymphocytes) and proliferating Ki-67+ cells. These cell populations were maintained the first two weeks and then gradually decreased.

In conclusion, it was possible to establish glioblastoma organoids from the three sub-regions of the tumor. The morphology, growth rate and cell composition were patient- dependent. This project sets the basis for a patient-relevant experimental system that can be used to determine effective drug combinations and response to radiotherapy in a patient-specific manner, but also to screen for anti-cancer therapeutics.

Reference

[1] Jacob et al. (2020). *Nat Protocols 15*, 4000-4033.

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Characterisation of primary NHP airway cells for MPS infection model development

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In the USA, the FDA has pioneered regulatory legislation which allows data generated in pre-clinical animal models to support product licensure. In addition, *in vivo* pre-clinical animal studies have enabled researchers to evaluate the pathogenicity of novel virus strains and variants to test novel drugs for efficacy and toxicity against them. As the SARS-COV-2 pandemic continues, the practicality of testing therapeutics, drugs and vaccines against all of the emerging variants of concern is no longer feasible. We believe that it may be possible to bridge animal *in vivo* and animal *in vitro* data.

In vitro human cell-based methodology such as organ-on-chip, microphysiological systems (MPS) and 3D tissue models offer potential alternatives to animal use. These technologies permit researchers to mimic the 3D structures of the human *in vivo* environment, including the initial immune response on-chip (Marzagalli et al., 2022). Despite this, many novel MPS models have yet to be rigorously tested and verified to the same degree as animal models.

Here, we present our progress in addressing the need to bridge the knowledge gap between animal *in vivo* studies and animal *in vitro* modelling methods for infection studies, using an animal *ex vivo* MPS model. Multiple lung cell types were harvested from the lobes and the trachea of post-mortem resected NHP tissues. Microvascular endothelial and alveolar endothelial cell types proved challenging to isolate and maintain *in vitro*. Alveolar epithelial and airway tracheal epithelial cells were successfully isolated and cultured. Tracheal cells were seeded directly onto transwells and reached confluence after five days. Transwell cultures were then

airlifted and cultured at the air liquid interface (ALI), and a differentiated mucociliary layer was achieved after approximately 21 days. Here, we present the results of characterisation studies on differentiated cultures using SEM and immunofluorescence.

Refinement of *in vitro* models is key to providing comparable infection data to that generated in animal models and may aid in demonstrating the suitability of MPS organ chip systems. This refinement may also assist in the reduction of animal use by helping to reduce our reliance on animal *in vivo* infection data.

Presentation: Poster

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Next-generation human iPSC-derived 3D brain systems to study chemical-induced myelin disruption and demyelinating diseases

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Growing evidence indicates that environmental toxicants contribute to the pathogenesis of neurodevelopmental disorders. However, the significance of exposure to xenobiotics during developmental stages is not fully understood. The Organization for Economic Co-operation and Development (OECD), defines toxicological test guidelines to evaluate chemicals and assure human health. However, developmental neurotoxicity (DNT) is not systematically studied due to the high costs, high number of animals, and time-consuming experiments required in the OECD test guidelines. In addition, there are rising concerns regarding the physiological relevance of extrapolating results from animal studies to humans. Together, this has resulted in a call for the development of a battery of New Approach Methodologies (NAM) assays that cover the most relevant key neurodevelopmental processes (KNDP) (e.g., proliferation, migration, myelination). Recently, an *in vitro* battery (IVB) was assembled from ten individual NAMs to investigate the effects of chemicals on various fundamental neurodevelopmental processes. However, some data gaps have been identified due to the absence of assays that address specific KNDP.

Myelination has been considered one of the most critical events during brain development and a sensible endpoint of DNT. However, the myelin assay in a human context, has not been incorporated yet in IVB of toxicity studies due to the difficulty to obtain myelin *in vitro*. Here, we used human induced pluripotent stem cells (hiPSCs) and generated a 3D model containing neurons and glial cells (also called BrainSpheres). This model presents compact myelin wrapped around axons, making it an ideal tool for myelin studies. Furthermore, it allows us to generate a reliable high amount of viable BrainSpheres, which are homogeneous in size and shape, and present reproducible percentages of the diverse cell types. To establish the myeline quantitative assay for high-throughput toxicological assessment and screening, we adapted our BrainSpheres protocol to make it suitable for High-Content analysis system. Myelin was detected by immunocytochemistry of myelin basic protein (MBP) and proteolipid protein 1 (PLP1). We aim to establish a standard operating system for automatized myelin quantification, after which a list of compounds ("training set") with either positive or negative effects on myelin will be used to validate the system.

Presentation: Poster

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Downscaled engineered heart tissues of entirely hiPSC-derived 3-cell-type co-culture are functional and viable over several weeks

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Microphysiological systems consisting of multiple cell types of the human heart have been shown to recapitulate certain aspects of human physiology better than conventional 2D *in vitro* models [1]. Engineered heart tissues (EHTs) that self-organise into contractile 3D structures between two flexible pillars are particularly useful to measure contraction against a force. However, conventional EHTs typically require between 50,000 and 2,000,000 cells, which makes creating many EHTs for high throughput screening costly [2]. Here, we show that downscaling EHT size, in our case to include human-induced pluripotent stem cell-derived cardiomyocytes (70%), cardiac fibroblasts (15%) and cardiac endothelial cells (15%), is feasible using as few as 16,000 cells. Tissues of three different sizes formed as expected and consistently, with 47,000, 31,000, and 16,000 cells. Moreover, while keeping the load constant relative to the size of the tissue [3], there was no difference in the viability nor functionality up to 14 days after formation. Electrical pacing of the tissues was conducted within the range of 1 to 3 Hz and with an optimal pacing frequency of 1.4 Hz, which is consistent over the three EHT sizes. Our results indicate that downscaled EHTs might be used as a cost-effective alternative to larger EHTs in drug discovery.

References

[1] Giacomelli, E. et al. (2020). *Cell Stem Cell 26*, 862-889.
[2] Mills, R. J. et al. (2017). *PNAS 114*, E8372-E8381.
[3] Dostanić, M. et al. (2020). *J Microelectromech Syst 29*, 881-887.

Presentation: Poster

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Development of a defined inflamed *in vitro* adipose tissue-lung model to investigate the influence of obesity on lung diseases

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Obesity describes a pathological level of fat reserves accumulated in the body. A chronically inflamed adipose tissue is a common side-effect of obesity. Especially in industrialized countries, the percentage of people affected is increasing dramatically. While many people are aware of the connection between obesity and, for example, diabetes, knowledge of the relationship between obesity and lung function is less widespread. However, it is known that obesity is a key risk factor for various lung diseases. In addition, existing obesity increases susceptibility to a variety of respiratory infections, and hospitalization rates for respiratory diseases in obese patients are higher than in patients with normal weight. Current studies also clearly show how obesity and severe courses of SARS-CoV-2 infection are related. To gain better understanding of the interplay of inflamed adipose tissue and lung diseases and infections suitable *in vitro* tissue models are needed.

We developed an inflamed adipose tissue consisting of mature adipocytes in co-culture with the monocyte cell line MonoMac6 and a lung tissue model consisting of epithelial cell line A549 and endothelial cell line EaHY926. For both models, a defined cell culture medium was developed. We were able to show appropriate cellular morphology and protein expression in the defined cell culture medium. To investigate the impact of inflamed adipose tissue on lung tissue the two tissue models were transferred into a perfusion cell culture system and the defined cell culture medium was adapted for the co-culture of all four cell types. We were able to demonstrate the impact of the inflammatory status of the adipose tissue on lung tissue concerning permeability, morphology, surfactant protein expression, and SARS-CoV-2 infection associated protein expression (e.g., ACE2, TMPRSS2).

The established defined inflamed *in vitro* adipose tissue-lung model in perfusion culture represents a promising tool to investigate the impact of obesity-related chronic inflammation of adipose tissue on lung diseases and infections.

Presentation: Poster

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On-chip maturation and characterization of iPSC-derived Langerhanoids

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Type 1 diabetes (T1D) results from immune-mediated β cells destruction in islets of Langerhans leading to absolute insulin deficiency. Stem cells (SCs) represent potentially unlimited source of new insulin producing β cells for T1D cell replacement therapy and disease modeling. The SC-derived β cells have been successfully obtained *in vitro* through stepwise differentiation process, but improvements in functional maturity are still needed. In regards, advances in culture systems such as microfluidic perfusion systems could enhance SC-derived islet functionality by providing biomimetic microenvironment in dynamic conditions. Here, we present generation of induced pluripotent stem cell (iPSC) derived islet organoids (i-Langerhanoids) using 2D and 3D models and microfluidic perfusion model that enables functional studies on one single i-Langerhanoid. Consequently, iPSCs were differentiated into pancreatic endoderm in planar culture by following 7-stage protocol [1]. Further differentiation into β cells was carried in 3D microwells culture that enabled enrichment of pancreatic progenitors and formation of uniformly sized aggregates. The i-Langerhanoids obtained were positive for β cell specific markers, but insulin secretion levels were lower compared to human cadaveric islets. To further characterize and improve β cell response to glucose, organoids were transferred to microfluidic perfusion chip. The microfluidic serpentine-shaped channel was designed previously with U-cup region dedicated to trapping single islets with diameter of 200-400 µm [2]. Here we have shown successful automated entrapment of i-Langerhanoids using hydrodynamic resistance on the chip where the effect of static and dynamic culture will be studied. Next steps include integration of functional vascularized organoids-on-chip by adding endothelial cells [3]. This will provide beneficial cues for islet growth, structure, and function, but also enable long-term culture. Improvements of physiological function will be monitored by glucose stimulated insulin secretion assay on-chip. Overall, combination of different cell culture systems and inclusion of heterogeneous cell types could bring SC-derived β cells closer to native pancreatic islets and provide model to study interactions underlying T1D.

References

[1] Fantuzzi, F. et al. (2022). *Front Cell Dev Biol.*
[2] Quintard, C. et al. (2022). *Biosens Bioelectron.*
[3] Quintard, C. et al. (2021). *bioRxiv*.

Presentation: Poster

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Reproducible creation of patient-specific cell lines from a wide variety of primary cells

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A major limitation of current research is the shortage of functional personalized cells. To generate cells in sufficient numbers for application in microphysiological systems, *in vitro* cell expansion is an attractive alternative. Cell expansion can be achieved by expression of immortalization genes. Establishing new cell lines is, however, an unpredictable and cumbersome process. In addition, conventional immortalization regimens often lead to drastic alterations of cellular physiology.

To overcome these limitations, we established a novel cell immortalization approach to reproducibly generate functional and immortalized cell lines. This approach relies on a custom library of more than 30 immortalization genes and allows efficient and reproducible creation of novel cell lines. We used this process to reproducibly establish cell lines from more than 20 different cell types and different individuals.

Single cell lines were generated within 2-3 months and included e.g., endothelial cells, astrocytes, smooth muscle cells, chondrocytes, kidney epithelial cells, thyroid epithelial cells, lung, and intestinal epithelial cells. Resulting cell lines are immortalized and can be cultivated for more than 100 cumulative population doublings. They can be easily cryopreserved and are amenable to genetic engineering. Functional characterization demonstrated that the novel cell lines maintain a primary-like phenotype and show cell type-specific functions.

Once an immortalization regimen was established for a certain cell type, it could be easily adapted for the reproducible immortalization of the same cell type from a different donor paving the way for individualized, patient-specific cell lines.

Novel cell lines responded to 3D culture conditions (e.g., matrix-free spheroids, Air-Liquid-Interface, semipermeable membrane) with an improved overall phenotype demonstrating their suitability as a reproducible and functional cell source for microphysiological systems.

This novel immortalization approach reproducibly provides personalized cell systems in sufficient numbers for even the most demanding applications.

Presentation: Poster

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Building a multi-tissue microfluidics system of metastatic potential (BIOMEP)

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Advanced prostate cancer (PCa) spreads towards bone, lymph node, and visceral organs, including liver. Preferential development of metastasis in specific organs indicates that invasion and colonisation of circulating tumour cells (CTCs) are strongly influenced by the organ host microenvironment, which will likely also impact therapy response. Hence, it is highly relevant to apply early drug screens in models that reflect clinically relevant metastatic sites. To reach this objective, we are aiming at replacing current models, including animal systems, by a humanised 3D culture system that allows for studying metastatic invasion of CTCs into different human healthy micro-tissues, such as liver and bone.

We started testing metastatic invasion into healthy human liver and bone models under static conditions as benchmark. As potential liver models, we compared i) human liver organoids derived from healthy liver biopsies, ii) primary human 3D liver microtissues (MCTs) consisting of a hepatocyte-Kupffer cell co-culture with cells obtained from multiple human donors and iii) spheroids generated from an immortalized human cell line (HepG2). Comparisons of expressed RNA and secreted protein levels indicate that the primary MCTs represent human liver function best while also enabling PCa cell invasion. As healthy bone model, mineralized microtissues of MG63 cells were used.

In parallel, we developed an advanced design of the Akura iFlow microfluidic system, which enables the continuous recirculation of suspended cells (PCa) and their interaction with 3D microtissues. Different PCa cell lines are being screened for viability and for their even distribution across the iFlow system by adapting medium composition, tilting parameters, and seeding density. Concomitantly, medium optimization is ongoing for co-culturing of both healthy microtissue models in the chip for two weeks.

Using the fully humanized 3D microfluidics system, we aim at identifying tumour cell and tissue factors that determine cell invasion into healthy tissues, which may guide metastasis-targeted drug development. Importantly, the generated knowledge may be transferred to other cancer types.

Presentation: Oral

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Patient-specific head and neck tumor microenvironment models for stratification of treatment efficacy

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Introduction: Improved prediction of treatment outcomes for cancer patients would greatly increase survival rates. This need is particularly acute in head and neck cancer (HNC), as survival rates in advanced disease remain poor and there are no clinically approved biomarkers. Better predictive tools such a patient-specific tumor microenvironment (TME) MPS constructed from cells isolated from the same patient, could stratify patients to targeted therapies while reducing the burden of systemic toxicities and could potentially offer dose-de-escalation where appropriate.

Methods: To create a patient-specific model of the head and neck TME, tumor tissue from each patient was digested to a single cell suspension. Tumor infiltrating leukocytes (TIL) were isolated using CD45 magnetic beads which were cryogenically stored after removal of the beads. The remaining cell types including epithelial cells and fibroblasts were isolated using specific media formulations and expanded in culture. A media formulation that supported the growth of all of the cell types in the chip was optimized using a high throughput microfluidic plate-based screen. An MPS based on the LumeNEXT microfluidic device was constructed using a hydrogel matrix containing TIL, fibroblasts, and an epithelial spheroid to model the solid tumor. Lumens molded from hydrogel were seeded with blood and lymphatic endothelial cells to create vasculature. Each patient-specific MPS will be treated with radiation and chemoradiation to mimic standard of care therapy and individual treatment responses will be measured using metrics of tumor spheroid growth, viability, angiogenesis, gene expression and cytokine secretion.

Results: We successfully created a patient-specific model of the head and neck TME using multiple components of the head and neck TME isolated from a single piece of tissue from each patient. We identified a media formulation that can support the viability of all 5 cell types in the MPS. The tumor spheroids show evidence of hypoxia in the core and continue to proliferate within the MPS. Importantly, the isolated cells retain patient-specific characteristics and differential responses to radiation treatment. This MPS has strong potential for testing patient-specific response of different classes of therapy including targeted therapies, i.e., immunotherapy, in addition to chemo/radiation or anti-antiangiogenic treatments.

Presentation: Oral

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The 3Rs Collaborative's MPS initiative: Collaborating to accelerate adoption of MPS in scientific research

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The 3Rs Collaborative's (3RC) Microphysiological Systems (MPS) Initiative is a collaboration of primarily commercial developers of MPS along with several MPS consultants, end-users, and other key stakeholders. This initiative sits within the larger 3Rs Collaborative which is a leading non-profit organization with the mission of advancing better science, for both people and animals. This mission is achieved by facilitating collaborative 3Rs opportunities (refinement, reduction, and replacement) to make a positive impact for people and animals in research and teaching using evidence-based science. The 3RC MPS Initiative was established to provide a venue for appropriate cross-developer & stakeholder collaboration to promote widespread adoption and regulatory acceptance of MPS.

This talk will provide an overview of the 3RC MPS Initiative's progress, outreach, and goals. It will present our Technology hub, a database of commercial MPS providers with tools to have an overview of available technologies and compare them, and to facilitate end-user engagement with MPS. The series of organ-specific workshops we have advanced with end-users and other stakeholders will be reviewed along with the contributions the initiative has made to general education about MPS. Finally, our engagement with regulators and our upcoming regulatory perspective paper will be discussed. Overall, this collaborative group hopes to boost the adoption of MPS as well as encourage standardization and regulatory acceptance. Through the 3RC MPS Initiative's collaborative efforts, we are accelerating scientific knowledge and contributing to humane animal research through refinement, reduction, and replacement of animal models.

Presentation: Oral

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Identification of anti-angiogenic activities of drug candidates by a perfused 3D angiogenic sprouting assay in Mimetas OrganoPlates

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Tumor angiogenesis is a hallmark of cancer progression. Consequently, the inhibition of angiogenesis has been demonstrated to effectively reduce progression of solid cancers, either by specific targeting of angiogenesis or as accessory activity contributing to the overall effectiveness of anti-cancer drugs. In addition, anti-angiogenetic therapy has proven very successful in several other diseases such as retinopathies. In contrast, unintended anti-angiogenic effects can cause severe toxicity in various tissues particularly during development and adolescence of organisms.

The organ-on-a-chip company Mimetas has developed a perfused 3D angiogenic sprouting assay [1] and previously showed its ability to phenotypically identify compounds with anti-angiogenic activity. We have adopted this assay to our existing lab environment and evaluated its potential to investigate anti-angiogenic activities of drug candidates. We selected a set of reference compounds acting via different molecular mechanisms. This test set was extended by compounds from early drug pipeline projects comprising proprietary molecules with expected anti-angiogenic activity and one compound with for which anti-angiogenic activity was hypothesized based on histological findings in an early systemic toxicity study in rats. All compounds were similarly tested for their anti-proliferative activity in 2D cultures of human umbilical vein endothelial cells (HUVEC). This study demonstrates how microfluidic assays can successfully be utilized during the drug discovery phase of the pharmaceutical R&D process. By comparing results obtained in the perfused 3D angiogenic sprouting assay with that in a conventional 2D anti-proliferation assay we will in addition highlight the importance of applying perfused phenotypic 3D models.

Reference

[1] van Duinen, V., Zhu, D., Ramakers, C. et al. (2019). Perfused 3D angiogenic sprouting in a high-throughput in vitro platform. *Angiogenesis 22*, 157-165. doi:10.1007/s10456-018-9647-0

Presentation: Poster

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MPS in safety assessment for DART and endocrine disruption: An industry perspective

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Encouraged by the successful application of New Approach Methodologies (NAMs) in an exposure-driven Next Generation Risk Assessment (NGRA) approach in systemic toxicity (Baltazar et al., 2022; Middleton et al., 2022) we created a framework to include additional *in vitro* assays covering endpoints for developmental and reproductive toxicity (DART) testing. NAMs from this framework comprised *in vitro* physiologically based kinetic (PBK) modelling, a cell stress panel, high-throughput transcriptomics, a DART extended set for *in vitro* pharmacological profiling as well as the ReproTracker® (Toxys) and the devTOX quickPredict™ assay (Stemina) to account for developmental toxicity. To determine if this extended set of NAMs is fit for purpose in ensuring sufficiently protective consumer safety assessments, its biological coverage was evaluated. Comparing cellular processes, signaling pathways and genes involved in known key stages in human reproduction and embryo-fetal development from an automated literature extraction to the read-outs from our NAM toolbox (including basic expression levels of cell lines) showed a more than 80% coverage on gene numbers (Rajagopal et al., 2022). Together with the evaluation of the framework using ~40 benchmark compounds certain areas could be identified where the use of MPS systems could be beneficial to enable human-relevant safety decision. These areas include placenta transfer models allowing the calculation of fetal exposure levels for an *in vitro* based PBK modelling strategy, differentiation of iPSC into additional cell lineages or organoid structures as well as multi-organ systems to evaluate endocrine signaling to fill existing gaps in the framework or for the refinement of the results. While many of these advanced cell systems already exist in biology only few of them are used routinely in toxicology. Therefore, the continued development and application, but also the robust evaluation of MPS in a decision-making context will play an increasing role in fulfilling the ambition to assure the safety of novel ingredients for human health for DART/endocrine effects without the need for animal testing. This presentation will present our ideas relating to where and how to integrate MPS systems into a NGRA framework as well as our initial results on some of the models tested to date.

Presentation: Poster

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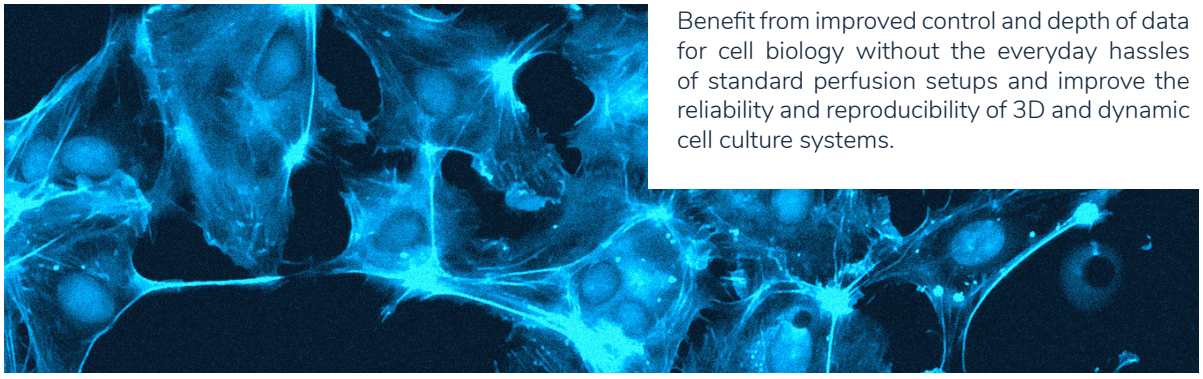

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A highly-sensitive integrated capacitive sensor for contractile force measurement in an engineered heart tissue platform

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Engineered heart tissues (EHTs) showed great potential in recapitulating tissue organization and function of the human heart *in vitro* [1]. Contractile kinetics is one key hallmark of cardiac tissue function and maturation level of cardiomyocytes, and a critical readout from EHT platforms. Typically-used optical methods to track elastic micropillar displacement upon tissue contraction are laborious and in most cases not conducted in real-time. This hampers automation and precise control of the EHT microenvironment. We address these unmet needs by developing a co-planar capacitive displacement sensor for tissue contraction force measurement integrated within an EHT platform.

The working principle of the displacement sensor relies on the deformation of the substrate wherein the sensors are integrated. Bending of each micropillar, caused by tissue contraction, results in local anti-symmetric out-of-plane deformation of the substrate. Two spiral capacitors are integrated below each micropillar of a previously developed EHT platform [2] to exploit the maximum substrate deformation.

The capacitive sensors were fabricated using a combination of wafer-level micromachining and polymer processing. The mould for the micropillars and elliptic well was fabricated by deep reactive ion etching of a Si wafer. Another Si wafer was covered with an 80 µm-thick polydimethylsiloxane (PDMS) layer, whereupon sputtered Al was photolithographically patterned into sensor designs. De-moulded micropillars and wells were aligned and bonded to the wafer with sensors. Single 10 x 10 mm^2 PDMS chips with integrated sensors were wire-bonded to custom-designed printed circuit boards. Analog Device AD7746 was selected to readout the expected aF-range change in base capacitance.

Static characterization of the sensors showed good agreement between measured and FEM-simulated values of base capacitance. The dynamic behavior was tested using a nanoindentation setup by applying specific force at different positions along the micropillars length while measuring the electrical response. Responsivity of 0.35 ± 0.07 fF/µN was measured. Preliminary experiments with EHTs proved the biocompatibility of the new platform with integrated sensors, as tissues were functional and in culture for at least 14 days.

References

[1] Giacomelli, E. et al. (2020). *Cell Stem Cell, 26*, 862-889.
[2] Dostanić, M. et al. (2020). *Journal of MEMS 29*, 881-887.

Presentation: Poster

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Human-based placenta-embryo chip for developmental toxicity assessment of nanoparticles

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Nanoparticles (NPs) are emerging materials enabling a wealth of novel applications, but little is known about their effects during pregnancy. Understanding developmental toxicity of NPs is indispensable to protect pregnant women and the developing fetus as well as to support the study of novel safe nanomedicines. However, there is a lack of physiologically relevant human *in vitro* models to study NP transfer and effects along the maternal-embryonic axis.

The aim of our project is to develop a fully human-based microphysiological platform, which recapitulates systemic effects of the maternal-fetal interface by realizing a placental barrier model in close vicinity to an embryonic model. Such a dynamic co-culture configuration is a prerequisite to capture not only direct embryotoxic effects from translocated NPs but also indirect effects from NP interference with placental functions and signaling factors that are essential to embryo-fetal development.

We have previously established a user-friendly microphysiological system that combines a placental barrier (BeWo trophoblast cell line) on a microporous membrane with murine embryoid bodies cultivated in a subjacent hanging drop [1]. To achieve a more predictive placental model, we replaced the BeWo cell line with a co-culture of primary cytotrophoblasts (CTBs) isolated from human term placenta and human placental vascular endothelial cells (HPVEC). We established a confluent co-culture barrier and verified spontaneous syncytialization of the CTBs. To verify the predictive value of the model for NP transport studies, we are studying size-dependent translocation of differently sized polystyrene NPs under static versus dynamic conditions and comparing the results to those obtained with a human *ex vivo* placenta perfusion model. As next steps, we will integrate a 3D embryoid body, derived from human induced pluripotent stem cells (iPSCs), in our microfluidic platform and verify developmental toxicity of selected NPs previously shown to be associated with adverse pregnancy outcomes.

The microphysiological placenta-embryo model will enable to gain novel mechanistic insights important to design safer NPs, to

protect pregnant women, and contribute to the reduction of developmental toxicity studies in animals.

Reference

[1] Boos, J. A. et al. (2021). Microfluidic co-culture platform to recapitulate the maternal – Placental – Embryonic axis. *Adv Biol 5*, 1-12.

Presentation: Oral

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The development of an intestinal organoid monolayer model to predict oral drug disposition

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The intestine has important gate-keeping functions that can profoundly impact the systemic blood exposure of orally administered drugs. Despite its importance, currently used *in vitro* models of the human intestine all have limitations which hinders their ability to broadly and accurately predict the disposition of such drugs. Due to these shortcomings, there has been an effort to develop advanced *in vitro* systems that more faithfully recapitulate *in vivo* drug metabolic enzyme and transporter (DMET) expression and activity. A promising complex *in vitro* system of the human intestine that can potentially fill this research need are intestinal organoid, or enteroid, monolayers, which are stem cell-derived, multicellular structures which recapitulate intestinal functions. While there has been significant growth of enteroid-related research over the past decade, the use of this *in vitro* model towards the application of oral drug pharmacokinetics is understudied. To address this knowledge gap, we investigated the utility of human enteroid monolayers to simultaneously assess intestinal drug absorption and first-pass metabolism processes. We cultured enteroid monolayers, collected via duodenal biopsies, from three donors and confirmed enteroid differentiation via RNA-seq analysis and immunocytochemical (ICC) assays. Proper cell morphology was assessed and confirmed via bright field microscopy and ICC imaging of tight junction proteins and other apical and basolateral localized proteins. Enteroid monolayer barrier integrity was demonstrated by elevated transepithelial electrical resistance (TEER) that stabilized after 10 days in culture and persisted for 42 days. These results were corroborated by low paracellular transport probe permeability at 7 and 21 days in culture. The activities of a prominent drug metabolizing enzyme, CYP3A4, as well as a drug efflux transporter, P-glycoprotein, were assessed and confirmed at 7 and 21 days in culture under basal, inhibited, and induced conditions. The duration of these experiments is particularly noteworthy, as this is the first study assessing DMET function for enteroids cultured for greater than 1 week. The sum of these results suggests enteroid monolayers are a promising *in vitro* model to predict the disposition of orally administered drugs and can be readily adapted for culture in microphysiological systems.

Presentation: Poster

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Tailoring human macrophages from iPSC for next generation MPS-based screening of immunotherapies

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Macrophages are innate immune cells with vital functions in the human organism. Given their important role in plethora of diseases and their remarkable plasticity, macrophages are of great therapeutic interest to develop new kind of immunotherapies. Screening and developing for such macrophage targeting therapies is, however, a challenging procedure, given the lack of an efficient *in vitro* model that can fully reflect the phenotype of a mature human macrophage in a high throughput approach. Especially the latter is of great interest, where modern e.g. micro physiological systems (MPS) can be further advanced with the addition of highly standardized human immune cells (e.g. macrophages). To bridge this gap towards more complex MPS, we here introduce the scalable production of human monocyte/macrophage (iMonoMac) from human induced pluripotent stem cells (iPSC). iMonoMac are fully defined and can be produced in reproducible quality and quantity from the same donor. Produced iMonoMac show a typical macrophage-like morphology and express typical surface marker: CD45, CD14, CD163, and CD11b. The production pipeline can be subjected to scalable systems, using either small (3ml), intermediate (50ml), or large-scale (250ml) devices. To use human sensor iMonoMac in complex MPS systems, we generated a genetically engineered iPSC line with stable expression of the NF-kB transcription factor coupled to a luciferase reporter gene (iMonoMac$^{NF\text{-}kB\text{-}Luc}$), able to sense pro/anti-inflammatory cues. Generated iMonoMac$^{NF\text{-}kB\text{-}Luc}$ were responding to external

pro-inflammatory stimuli and were highly sensitive to a dose-escalation of LPS with a minimum detection of 3.3 pg/mL at 6 h post incubation. Of note, iMonoMac$^{NF\text{-}kB\text{-}Luc}$ showed high specificity with low background luminescence in a non-activated state. To lay the foundation for a macrophage-cancer-MPS system, we utilized the activity of iMonoMac to respond to state-of-the-art phagocytosis check point inhibitors. Indeed, a well-known anti-CD47 antibody was able to enhance the phagocytic activity of iMonoMac targeting either leukemic (Raji) or adenocarcinomic human epithelial (A549) cells by 2- or 1.5-fold respectively after 2 h of coculture. In summary, highly standardized and custom-made human immune cells from iPSC provide a unique tool to establish next generation MPS based screening modules, paving the path for developing future immunotherapeutics.

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Engineering and investigating neural circuits *in vitro* on high-density microelectrode arrays

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The vast complexity of neural circuits *in vivo* hinders the investigation of memory formation and information processing in the brain. Gaining insights into the fundamental mechanisms behind neural and synaptic behavior is necessary to advance our knowledge in fundamental brain functionality and to accelerate the drug discovery process for neurological disorder and disease treatment.

To tackle these challenges, we suggest the use of engineered neural circuits formed on microelectrode arrays (MEAs) as a platform to study neuronal activity in parallelized networks of highly reduced complexity [1]. This bottom-up neuroscience approach makes use of polydimethysiloxane (PDMS) based microstructures that constrain the number of cells and provide guidance cues to promote directional axon growth between several neural populations. The network topology can be designed in various ways by adapting the microstructure design to different experimental needs.

We demonstrate a platform that allows the formation of such networks on high-density MEAs consisting of 26,400 recording sites using primary rat neurons and human induced pluripotent stem cell (hiPSC) derived neurons. We show that we can culture engineered neural networks for several weeks *in vitro* and obtain signals with large signal-to-noise ratio across the whole network. The high electrode density allows to study the propagation of information with high spatial resolution down to subcellular features [2]. Moreover, the use of high density MEAs allows to apply a stimulus to every electrode underlying the network. We demonstrate that we can track the propagation of stimulation-induced activity within networks of various topologies and can selectively trigger different activity pathways within the same network.

The use of such topologically constrained networks on high density MEAs allows to study their input-output relationship with unprecedented precision. We believe that this platform can be used to gain insights into how neural networks form and how they process information and will benefit research in systems, computational and translational neuroscience.

References

[1] Ihle et al. (2022). An experimental paradigm to investigate stimulation dependent activity in topologically constrained neuronal networks. *Biosens Bioelectron*.

[2] Duru et al. (2022). Engineered biological neural networks on high density CMOS microelectrode arrays. *Front Neurosci*.

Presentation: Poster

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Robust workflows for the expansion and differentiation of human pluripotent stem cells as aggregates in suspension culture

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STEMCELL's TeSR™ 3D-based media products have been developed for robust and scalable suspension culture of human pluripotent stem cells (hPSCs). Despite a large range of systems available from other cell types, the field has been challenged by the lack of methods to reproducibly scale hPSC suspension cultures beyond small volumes. One challenge is related to the requirements of mixing, where sufficient agitation should be maintained to reduce aggregate fusion but shear must be minimized to avoid damage to the aggregates.

6 hPSC lines were serially expanded in 9 different suspension culture vessels including 6 well plates on orbital shakers, orbital shaker bottles and vertical wheel PBS-MINI Bioreactors. Aggregates were passaged non-enzymatically using Gentle Cell Dissociation Reagent and filter-based trituration. A workflow was identified that could scale the hPSCs from 2 mL to 500 mL. hPSCs

underwent ~ 1.5- to 1.9-fold expansion per day (cell line-dependent) with > 85% viability, > 90% expression of OCT4 and TRA-1-60. The aggregates maintained their capacity to differentiate to the three germ layers, while demonstrating uniform undifferentiated morphology and a normal karyotype. The scalable, low-shear Vertical-Wheel™ impeller design yielded reproducible growth across cell lines at the largest growth volumes. To demonstrate the downstream applications of the suspension workflow using the Vertical-Wheel™ bioreactor, 2 hPSC lines were differentiated into polyploid differentiation (MKs) in suspension cultures. Differentiation used a 12-day endothelial-to-hematopoietic transition phase and a 5-day progenitor-to-mature MK stage. At the end of the protocol, 55-90% of cells expressed CD41a, 40-80% of the cells co-expressed CD41a and CD42b, and 15-60 CD41a+CD42b+ cells were generated per seeded hPSC (n = 13). The DNA ploidy profile of the CD41a+CD42b+ cells generated showed 26% and 9% of cells had 4N and 8N+ DNA ploidy, respectively.

The combination of TeSR™ 3D-based media products and low-shear bioreactors provides a robust system for the expansion of a wide range of hPSC lines. With robust workflows in place, focus can be shifted to differentiation in suspension culture for cell therapy.

Presentation: Poster

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Differential susceptibility of gastrointestinal epithelium monolayers to SARS-CoV-2

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Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) is a global health problem and a cause of coronavirus 2019 (COVID-19) pandemic. Although the majority of symptoms of the disease manifest as a respiratory complications, clinical and experimental data showed the virus can infect a broad range of tissues, including the epithelial lining of the gastrointestinal tract. Here, using adult stem cell-derived monolayers, we examine the susceptibility of gastrointestinal epithelium to SARS-CoV-2 infection. Data shows that while small intestine and colon is susceptible to SARS-CoV-2 infection, gastric epithelium seems to be protected from infection. This is mirrored in the expression of the known SARS-CoV-2 entry receptor, ACE2, in gastrointestinal tissue, organoids and 2D monolayers using RNA sequencing, mass spectrometry and immunofluorescence. ACE2 expression was highest in small intestinal jejunum, intermediate in colon, and almost absent in gastric corpus. To verify, whether ACE2 was indeed the central limiting factor, we overexpressed in gastric organoids. Data showed increased SARS-CoV-2 replication in gastric organoid-derived monolayers overexpressing ACE2. In summary, our data shows a differential susceptibility to SARS-CoV-2 in gastrointestinal epithelium, mediated by differential expression of ACE2, allowing the virus to preferentially infect small intestinal cells and promoting an enteric stage of the virus infection.

Presentation: Poster

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Microfluidic platform for transwell-based upper airway cultures under alternating air-liquid and liquid-liquid interface conditions

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The global antimicrobial-resistance threat promotes the development of novel models of the human upper airway for pulmonary disease modeling. Conventional transwell insert-based membrane models have become increasingly popular with recent advances in stem cell technology [1]. Various parts of the respiratory epithelium were established in transwells and have been shown to exhibit *in vivo*-like cell morphogenesis and functional heterogeneity. A permeable membrane at the bottom of the transwell enables the establishment of an air-liquid interface, which recapitulates an *in-vivo*-like state. However, the investigation of complex tissue architectures and cell-to-cell interactions at high temporal and spatial resolution is still challenging within a conventional transwell insert. Real-time high-resolution imaging is significantly limited as long-working-distance objectives are required. The apical side of the epithelium is usually exposed to ambient air and kept free of liquid media. Such a configuration entails significant limitations for regular washing steps of the apical surface for clearing excess mucus and collection of the apical cell secretome.

Here, we present a modular organ-on-chip system that enables long-term culturing and imaging of upper airway tissue on transwell inserts in a microfluidic device. The microfluidics allow for

controlled perfusion of liquid medium at defined time intervals, which facilitates a controlled and seamless transition between air-liquid and liquid-liquid states at the tissue. This repeated alternation enables to maintain tissue viability and integrity over a prolonged time, while enabling a regular sampling at discrete time points from the apical side for analysis of airway-specific cytokines and chemokines. The system is compatible with high-resolution imaging and enables continuous monitoring of the tissue model. Finally, the device was fabricated in biocompatible poly(methyl methacrylate) (PMMA), which helps to overcome the problems of compound absorption and renders it an attractive system for quantitative *in-vitro* drug testing studies.

This work was financially supported by the Swiss National Science Foundation (SNSF) within the framework of the National Competence Center in Research "Antiresist", New approaches to combat antibiotic-resistant bacteria under contract number 51NF40_180541.

Reference

[1] Shi, D. et al. (2019). In vitro and ex vivo systems at the forefront of infection modeling and drug discovery. *Biomaterials 198*, 228-224.

Presentation: Poster

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Microfluidically constructed novel brain-on-chip microenvironments

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Simplifying protocols for stem cell-derived neural networks [1] and characterizing neuron's function thoroughly, e.g. by applying microelectrode arrays, new ideas for advanced concepts for Brain-on-Chips (BoCs) emerge. Previously, we enabled extrinsic inputs by a mechanical stimulus using stacked hydrogels to mimic differences in brain tissue stiffness in our models [2].

3D printing is becoming popular for microfluidic fabrication considering that standard fabrication techniques are cost-intensive processes with complex and time-consuming operations. Moreover, 3D printing offers more design freedom, able to print intricate designs, reduction of waste products [3]. Inspired by the progress of 3D printing for Organ-on-Chip we replica-molded a microfluidic construct in polydimethylsiloxane (PDMS) that generates 3D microenvironments in combination with dispensed hydrogels beyond simple pipetting actions. Our mold was printed by a Form 3+ printer (Formlabs) using Clear v4 resin type. To ease PDMS release, Ease Release 200 (Mann Technologies) has been sprayed onto the mold surface from a 15 to 20 cm distance. After drying under the fume hood for 5 min, degassed PDMS mixture (10:1) was poured into the mold and cured at 65°C in the oven overnight. After peeling off, the PDMS layer was tested for its microfluidic function by sealing it to a glass microscope cover slip. Our fluidic design with microgrooves incorporated in the sidewalls of the channel allows us to pin a liquified gel in the z-direction while flowing along the channel upon insertion in one of the reservoirs at the channel endings. We can then tailor the culture configuration further by using these pinning grooves also for medium flow along the sidewalls of the channel during the operation of the BoC. Next, we will assemble the newly designed PDMS layer to the membrane layer of our actuator chip to provide instructive 3D microenvironments in progressing microphysiological systems studies.

References

[1] Gunhanlar, N. et al. (2018). *Mol Psychiatry 23*, 1336-1344.
[2] Akcay, G. and Luttge, R. (2021). *Micromachines 12*, 165.
[3] Carvalho, V. et al. (2021). *Sensors 21*, 3304.

Presentation: Poster

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Three-dimensional *in vitro* modeling of the subventricular (SVZ) neural stem cell niche

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Our purpose is to generate a suitable microenvironment for neural stem cells (NSCs), which will recapitulate *in vitro* the normal subventricular zone (SVZ) NSC niche physiology. Thus, by understanding both the physiological conditions as well as the pathophysiology can be used for the establishment of a pathophysiological model of congenital hydrocephalus (CH). Conventional cell culture and early tissue engineering methods suffer from limitations including limited distribution of biomolecules by diffusion throughout the engineered tissue, and lack of natural interactions and physicochemical cues between the ECM and the cells themselves. Although theses 3D techniques provide a better biomimetic microenvironment for cells and stem cells compared to two-dimensional (2D) cultures, they still have limitations and have encouraged researchers to further develop optimized methods. In our case, we have established a static transwell organotypic culture of the SVZ niche using region specific decellularized matrix, closely mimicking the native SVZ stem cell niche comprising of ependymal cells, radial glial cells (RGC), astrocytes and NSCs. Our pre-

liminary data suggest that further development and usage of a dynamic microfluidic culture would better imitate the physiological analog which requires a constant flow rate of the cerebrospinal fluid as it happens normally *in vivo*. Our ultimate goal is to establish a pathophysiological model of hydrocephalus, which will lead to translational applications for studying and developing personalized medicine protocols.

Presentation: Poster

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A high-throughput vascularized immunocompetent organoids on well: Application on immuno-oncology for breast cancer

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An increasing number of oncological patients is benefitting from the raising immuno-oncology arsenal available to clinicians. Nevertheless, a relevant portion of patients is not responding to treatments. The early identification of not-responding patients can have a direct impact on the prognosis of people candidates for immune-oncology treatments. One of key factors to better predict the effect of a therapy is the possibility to recreate, *in vitro*, the cellular and physical tumor-immune microenvironment (TME).

We here present for the first time a high-throughput vascularized immunocompetent breast organoids based on standard 24 MW plates. This micro-physiological system (MPS) was conceived to be able to observe cancer cells invasion and control/recreate, *in vitro*, the tumor microenvironment (TME) to evaluate treatment in the presence of cancer cells-cancer associated fibroblast-immunoresident interplay.

The physical microenvironment was established using Cherry Biotech's MPS control unit and multi-well adaptor, CubiX, in combination with 4DCell SmartSphero Plates (SSoP). SSoP ultra-low attachment hydrogels allows the formation of triple-negative cancer organoids made of monoculture of MDA-MB-231 cells in specific positions. The specific anchoring points of SSoP allowed us to add a collagen matrix laden with human primary fibroblasts and immune cells (CD81+, CD64+). The blood/ tissue endothelial barrier consisted of a monolayer of differentiated endothelial cells (CD31+).

We monitored the organoids growth rate using time-lapse imagining. Endothelium differentiation, presence, and distribution of CD81+ and CD64+ cells were assessed by immunolabeling.

All cell types were kept viable for 7 days, endothelial cells alignment was achieved and comparable to *in vivo* parameters.

This combined system, which to the best of our knowledge, is the only one able to recreate the TME in a 24 MW with multiplexed and uniform organoids sizes and positioning. The CubiX system controlled the cell culture conditions directly under the microscope providing nutrients, shear stress to differentiate endothelial cells, dissolved gasses, and stable temperature. Online sensors monitored the culture evolution by measuring lactate, glucose, O_2 consumption.

We envision that this model will evolve into a vascularized immunocompetent patient-derived tumor well and provide a new tool for assisting clinicians in finding the best therapeutic approach

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Generation of an alveolus-on-chip model for personalized drug screening against viral-bacterial co-infections in viral pneumonia

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Secondary bacterial infections are a frequent complication of influenza virus (IV) infections that can manifest as seasonal epidemics and occasional pandemics. To date, there are no specific medications for treating IV – bacterial super-infections. Animal models and conventional cell culture approaches have been frequently used to study the underlying pathogenesis of co-infections. However, existing *in vitro* techniques typically fall short of accurately capturing the complexity of the lung, while animal models frequently can't adequately represent the human lung. Thus, novel patient-specific test systems are urgently required to develop effective anti-infective treatments for secondary bacterial infections.

Existing lung-on-chip models mostly use immortalized cell lines or primary cells, which are both associated with inter-individual variability and the risk of allogeneic responses in immunocompetent models due to mixing up cell material from different donors. To overcome this limitation, human-induced pluripotent stem cells (hiPSCs) were differentiated into alveolar type II cells (AT2s) and complemented with isogenic iPSC-derived endothelial cells and macrophages. Using tissue engineering, we recreated the alveolar barrier with an air-liquid interface in a microfluidi-

cally perfused biochip. We were able to successfully infect AT2s with IV and used immunofluorescence microscopy to track cell type-specific progression of the infection process. By employing hiPSCs from various donors, we will evaluate novel medication options and the patient-specific response to viral-bacterial co-infections in the novel alveolus-on-chip model.

References
[1] Schicke, E. et al. (2020). *Microorganisms 8*, 4.
[2] Deinhardt-Emmer, S. et al. (2020). *Biofabrication 12*.
[3] Jacob et al. (2021). *Nat Protoc 14*.

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Long-term modular human iPSC-derived neuronal networks on-chip

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Neuronal networks in the brain are highly organized structures composed of various functional circuits. Bottom-up engineering approaches like 2D or 3D neuronal cell culture techniques attempt to model some parts of the intricate brain circuitry [1]. However, network structures of the *in vitro* neuronal cultures deviate between replicates and changes over time [2]. This makes it difficult to attribute a functional electrophysiology output to a specific network structure. Therefore, the generation of *in vitro* neuronal networks with defined microstructures are an essential approach to model brain circuits1. In the present work, a thin (100 µm) microchannel device was fabricated using polydimethylsiloxane (PDMS) to host neurons and guide axonal outgrowth. This device is composed of 64 microwells (8 x 8), for housing neuron somas and smaller connecting microchannels between these separate network modules only allowing neurites to grow through. The microfluidic device was placed on planar electrodes of standard multi-electrode array (MEA) chips to enable non-invasive long-term electrophysiology recordings from developing modular circuits. High-density and low-density modular networks were generated by seeding neurons in high and low densities within the integrated device. We used a well-established transgenic human induced pluripotent stem cell (hiPSC) line, the inducible neurogenin (iNGN) line, for network generation. In iNGN cells, a TetOn promotor system is used to induce the overexpression of the transcription factors Neurogenin-1 and Neurogenin-2 leading to rapid neuronal differentiation in four days [3]. Long-term functional features of low- and high-density engineered modular networks were compared to randomly formed control networks on MEA chips without a microchannel device. Our data show that in modular networks a higher percentage of neurons are accessible for functional recordings. The high-density group had strong, and longer synchronized burst activities resembling the developing prenatal human brain. Modular engineered circuits with controlled network structures and solid activity profiles over months offer a reliable *in vitro* testbed for studying brain circuits in health and disease.

References
[1] Habibey, R. et al. (2022). *Chem Rev 122*, 14842-14880.
[2] Habibey, R. et al. (2022). *Front Neurosci 16*, 951-964.
[3] Busskamp, V. et al. (2014). *Mol Syst Biol 10*, 760.

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From hiPSC-derived liver organoids to hiPSC-derived liver-on-chip: Enhancing cytochrome P450 expression for drug metabolism studies

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Introduction: An individual's response to common drug treatments varies between 50-75% and genetics play an important role herein. The cytochrome P450 (CYP) genes family is responsible for the biotransformation of 70-80% of all drugs in clinical use. To study the role of CYP variants in the drug metabolism process, human induced pluripotent stem cells (hiPSC) are used to generate a personalized *in vitro* model system with the preservation of the individual genetic makeup.

As the liver is the main organ for drug metabolism, optimal differentiation and maturation of hiPSCs to hepatocytes is essential. Although hiPSC-derived hepatocytes (hiHeps) express hepatocyte-specific markers (e.g. *Hnf4a*) and show functionality by albumin secretion, *CYP* expression remains low or undetectable. Here, we aimed to generate hiHep-derived liver-on-chip with enhanced *CYP* expression by including an organoid culture intermediate step, to study drug metabolism.

Materials and methods: hiPSCs were differentiated towards hepatic progenitor cells and seeded in Matrigel domes to form hepatic organoids. The organoids were expanded and characterized by qPCR and IF at each differentiation stage to assess the loss of "stemness" and the establishment of a mature phenotype. Once optimal conditions and time points were defined, the organoids were dissociated and seeded on-chip. A small drug panel was selected based on the CYP expression of hiHeps on-chip to prove

metabolic capacity, including drugs metabolized by *CYP3A4*, the most important drug-metabolizing enzyme.

Results: The organoid configuration provided pivotal cell-matrix and cell-cell interactions necessary for these cells to present a mature phenotype, including the expression of *Hnf4a*, *Aat* and *Mrp2* and albumin secretion and, importantly, enhanced *CYP* expression. The relative expression of CYP enzymes was compared to primary hepatocytes and immortalized hepatocytes on-chip and was proven to be sufficient to study drug metabolism on-chip.

Conclusions: Altogether, our data indicate that the generation of hiPSC-derived organoids is a key step in the development of a physiologically relevant personalized *in vitro* liver model to study drug metabolism on-chip. Future outlook includes the co-culture of multiple liver cell types and the extension of the hiPSC-based liver chip to a personalized microbe-gut-liver-on-a-chip, taking into consideration individual genetics and metagenomes and microbiome simultaneously.

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Autologous co-cultures of human intestinal CD8+ cells and organoids on-chip to recapitulate a mucosal immune response

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Celiac Disease (CeD) is a complex, multifactorial and immune-mediated disorder, characterized by a strong inflammatory response in the small intestine triggered by dietary gluten. The inflammation eventually results in the activation of CD8+ intraepithelial lymphocytes (IELs). The activated IELs attack and damage the intestinal epithelial cells (IECs), leading to villous atrophy. The molecular crosstalk between IELs and IECs driving the IEC destruction has however remained elusive. In this study we aim to elucidate these molecular drivers of villous atrophy in CeD by establishing a novel patient-derived immunocompetent mucosal barrier-on-chip model of the small intestine.

For this, we generated human intestinal organoids (HIOs) from duodenal biopsies taken from CeD patients and controls participating in the Celiac Disease Northern Netherlands (CeDNN) biobank. In parallel, we were able to isolate and culture CD8αβ+ TCRαβ+ IELs from a duodenal biopsy of the same individual what allows for the generation of a temporary patient specific IEL cell line. These cells were used to set-up viable long-term autologous co-culture systems of IELs. With this, we can recapitulate the IEL migration towards, and interactions with IECs by life cell microscopy. CeD relevant cytokines Interleukin 15 (IL-15) and IL-21 potentiate the IELs in co-culture, indicated by elevated Granzyme B (GzmB) and Interferon gamma (IFNγ) levels. Also, functionality (i.e. the cytotoxic potential) of the IELs towards the HIOs can be followed as demonstrated by measuring apoptosis of the organoids.

This new autologous co-culture system thus allows for monitoring key aspects of the CeD mucosal immune response. Currently we are performing side-by-side comparisons of patient-derived and control-derived co-cultures to identify differences in IEL activation and cytotoxic potential. We aim to do in depth characterizations of the IEL and IEC compartments separately using (single cell) RNA sequencing. This co-culture system will be further advanced by recreating the IEL-epithelial barrier interface in a microfluidic chip, which will provide a better controlled environment and allows integration of mechanical stimulation such as continuous fluid flow. Ultimately, this patient-derived model system will shed light on the molecular drivers of villous atrophy in CeD and can be used to test potential therapeutic interventions.

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A kidney-on-a-chip to study the role of hydrodynamic constraints in cyst formation in polycystic kidney disease

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Scattered loss of *PDK1* in Autosomal Dominant Polycystic Kidney Disease (ADPKD) locally disrupts the kidney tubules. Cells no longer expressing *PKD1* may lead to localized dilations of the nephrons, giving raise to cysts. Surprisingly, all gene loss events do not immediately lead to cysts formations. A slow-paced onset of cysts in the kidney early in life becomes exponential around 40-50 years old. Strikingly, cysts propagate in clusters due to ill-identified mechanisms. It may originate from mechanical interactions because of nephron proximity. Cysts collapse neighboring tubules, obstruct the physiological luminal flow and lead to abnormal intraluminal pressure. Motivated by a lack of understanding for their direct involvement, we are investigating the role of those mechanical constraints at the origin of tubular deformations.

Our perfusable kidney-on-a-chip mimics the geometry of several nephrons in a deformable collagen matrix with parallel tubules of 75 µm diameter as close as 100 µm apart. These tubules are

seeded with wild-type kidney cell lines and their derived ADPKD cell lines. We optimized the geometry of the microfluidic channels in the chip scaffold to allow a *long-term perfusion decoupling luminal pressure and flow*. It allows us to focus on these constraints separately or together.

Our results on two cell lines originating from distinct parts of the nephron showed that in static conditions, Pkd1KO tubules already experience an important dilation, *more than 1.5-fold their original diameter in 1-2 weeks*, contrary to control cells. Surprisingly, we observed that in cells originating from collecting ducts, these deformations depended on the stiffness of the matrix and were not driven by an increased proliferation but rather by an active remodeling of the matrix. The importance of luminal mechanical constraints is illustrated by our preliminary findings that *Pkd1KO tubules under luminal pressures as low as 10 mbar experience a strong dilation visible in 2 days only*, with or without physiological flow. At last, we are mimicking scattered loss of *PKD1* occurring in ADPKD by seeding Pkd1KO cells with control cells in a controlled ratio, and testing the influence of adding physiological or pathological hydrodynamic constraints to trigger cyst-like structures

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Building human induced nigrostriatal microcircuits on CMOS chips to study Parkinson's disease

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Despite the tremendous efforts made by clinical geneticists and epidemiologists, the cause of most Parkinson's disease cases remains unknown. We hypothesize that the molecular alterations that cause PD manifest in characteristic electrophysiological phenotypes in disease-relevant neuronal circuits. To test this, we are building synthetic nigrostriatal circuits on top of CMOS multielectrode array (MEA) microchips.

We have optimized the derivation of neural progenitors specific to the developing cortex, striatum, and ventral midbrain from induced pluripotent stem cells. These progenitors are seeded onto specific locations of the chips and primed to differentiate into their corresponding neuronal-striatal projection, cortical excitatory and nigral dopamine neurons- progeny. We engineered a triple fluorescent reporter system that enables us to monitor the birth of the different types of neurons and the establishment of physical connections through their axons and dendrites. Using the chip's electrodes, we are evaluating how the different neurons acquire electrophysiological and synaptic maturity. In addition, by applying a combination of receptor agonists and antagonists, optogenic actuators as well as enabling the electrodes to stimulate specific subsets of neurons, we can unravel the functional connectivity as the network develops.

Once we have characterized the properties of the synthetic circuits and their reproducibility, we will generate circuits from patients, phenotype them and ultimately identify functional similarities that will enable us to stratify PD in a series of mechanistic sub-clusters.

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The next generation of iPSCs alveolus-on-chip: Combine flexible collagen-I membranes, mechano-biological stimulation, and a human pneumonia model infection

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Introduction: Organ-On-Chip (OOC) stimulates the physiologically perfused environment of tissues. The majority of OOC systems contain membranes that are utilized as cell culture scaffolds and to mimic extracellular matrix (ECM) functions, which are critical for cell attachment, cell differentiation, and guidance of morphogenesis in development as a prerequisite of proper function [1]. Current membranes, made of materials such as polydimethylsiloxane (PDMS), however, have some significant disadvantages, such as non-selective absorption of hydrophobic compounds that could contribute to bias in drug testing studies [2].

Experimental procedure and results: In this study, we aim to replace existing artificial membranes with flexible Collagen-I membranes to establish an immunocompetent alveolus-on-chip model [3]. Human pluripotent stem cells (iPSC) generate lung alveolar type II, endothelial, and macrophage cells. The cell types were arranged in a bioinspired manner and co-cultured on a Collagen-I membrane. Immunofluorescence imaging was used to assess the viability, confluency, barrier formation, and expression of critical biological markers. In follow-up studies, a tissue engineering approach will create an isogenic alveolus-on-chip model.

Future perspective: This isogenic model will be employed in infection research with *Staphylococcus aureus* to investigate molecular mechanisms of pneumonia. The effects of simulated breath-

ing biomechanics and ventilation will be addressed using strain and osculating air pressure to assess functional tissue alterations throughout the infection.

References

[1] Zamprogno, P. et al. (2021). Mechanical properties of soft biological membranes for organ-on-a-chip assessed by bulge test and AFM. *ACS Biomater Sci Eng 7*, 2990-2997.

[2] van Meer, B. J. et al. (2017). Small molecule absorption by PDMS in the context of drug response bioassays. *Biochem Biophys Res Commun 482*, 323-328.

[3] Arık, Y. B. et al. (2021). Collagen I based enzymatically degradable membranes for organ-on-a-chip barrier models. *ACS Biomater Sci Eng 7*, 2998-3005.

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Investigation on the effect of ketogenic diet on human neurovascular unit-on-a-chip: Brain energy metabolism with different diets

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Ketogenic diet is one of the clinically validated treatments for refractory epilepsy and an effective treatment for a few other neurological disorders. This treatment is a shift from using glucose to ketone bodies/ fatty acids to provide an alternative energy source for the cells, particularly brain cells. In this study, first a functional blood-brain-barrier (BBB) on a microfluidic chip was established which the vascular channel of the chip was lined with primary human brain microvascular endothelial cells and the brain channel was lined with primary astrocytes. The evaluation of the functionality of the established BBB-on-a-chip was performed with different techniques including microscopy imaging and permeability assessments. Later, different mimics of diets (glucose and ketogenic diet) were introduced into the BBB-on-a-chip to study the passage of diets from vascular channel to brain side as well as the energy metabolism for astrocytes. It was shown that the mimic of the ketogenic diet composed of fatty acids results in ketone body (beta hydroxybutyrate) production by astrocytes which could serve as an energy source for neurons in the body. Moreover, using qPCR the gene expression assessment was performed for some key genes linked to the metabolic enzymes in which the upregulation of some key relevant genes was detected. Furthermore, using mass spectrometry data assessment, the metabolic pathway analyses were preformed to further explore the underlying mechanism of brain cells energy metabolism by the ketogenic diet. This study paves the way for using the ketogenic diet on organ-on-a-chip technology for modeling metabolic disorders such as epilepsy.

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Engineering of development-like tubulogenesis to construct non-embedded liver sinusoid on chip for mechanobiology studies

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The capillaries, the vascular endothelium lying at the vessel-parenchyma interface, plays a crucial role in the homeostasis and behavior of the surrounding tissue. These microvessels constitute a barrier, selectively limiting the passage of blood plasma, circulating cells and pathogens thanks to the endothelial cells, also responsible for the vessels' structural integrity. *In vivo*, their architectures are dynamically remodeled in response to external stimuli and disruptions occur in diseases related to mechanical stresses linked with obesity and fibrosis, especially in the liver. Indeed, hypertension, increased matrix stiffness or augmented blood shear stress impair the health of the hepatic tissues, although the mechanisms are not identified. Currently, we lack a truly physiologically-relevant model of perfusable microvessels not hydrogel-embedded.

Here, we propose a development-like cell self-organization strategy, to guide the emergence of a perfusable tubular endothelial microarchitecture that can recapitulate the barrier functions and flow dynamics of microvessels *in vitro*. In particular, we engineer free-standing, perfusable microvessels not embedded in fixed ECM gel, using liver sinusoidal endothelial cells (LSECs). This liver-sinusoid-on-chip (LiverSoC) faithfully recapitulates true liver sinusoids that present no basal membrane *in vivo* to study the disruption of their phenotype by such environmental mechanical stimuli.

We show that a developmental biology process can be engineered in a controlled way, using a LSEC cell line, to form structurally stable, lumenized tubes at the boundary between laminin-rich soft substrates and glass. We describe the step-by-step process where cells align and polarize in a cord along the boundary, before following progressive tubulogenesis in one week, around the cord. Confocal immunofluorescence and cell char-

acterization were performed over the course of 8 days, showing tube formation with local cell death and slow evolution of cell-cell junctions (ZO-1), cytoskeleton (actin) and polarization (podocalyxin) markers. We show this process depends strongly on the nature of the ECM and geometry employed and that the final tubular microstructure can be manipulated and interconnected to microfluidic pumps for perfusability and permeability assays. Finally, we show that the cells forming the structures conserve angiogenic potential, with the controlled formation of sprouts for further interconnections leading to tissue vascularization using our self-organized vessel

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Replicating organ-organ (BBB-brain) interaction with modular tissue-in-a-CUBE chip

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The interaction between organs is challenging to replicate *in vitro* because of the varying culture conditions of the various cell types involved. Furthermore, combining multiple tissues often requires complicated microfluidic setup, and fragile tissue samples do not fare well with rough handling. We previously developed a Tissue-in-a-CUBE device comprising a polycarbonate frame with agarose outer walls, in which cells can be cultured in a 3D extracellular matrix (ECM), allowing easy picking up of the sample with a pair of tweezers (Hagiwara et al., 2016). In this study, we present the development of a multi-organ chip that takes advantage of the modularity and convenient handling ability of the CUBE, with a demonstration of blood-brain barrier (BBB)-brain interaction.

We first developed a BBB-in-a-CUBE model by co-culturing primary astrocytes and pericytes with iPSC-derived brain microvascular endothelial cells in the CUBE. The expressions of important tight junction and transporters were confirmed by immunofluorescence staining and RT-qPCR. We also devised a polydimethylsiloxane (PDMS) chip to analyse the trans-endothelial electrical resistance (TEER), as well as Lucifer Yellow and Rhodamine123 permeability, of the BBB model.

As proof-of-concept, we prepared a Brain-in-a-CUBE with T98G glioblastoma cells in the CUBE as a brain cancer model. At the appropriate timing, BBB-in-a-CUBE and Brain-in-a-CUBE were integrated in a chip designed to hold two CUBEs with separate media chambers. Vincristine, a P-glycoprotein (PGP) substrate glioblastoma drug, was added to the BBB side, and T98G analysed by live/dead assay. Treatment with vincristine when a BBB was present resulted in less cell death compared to without BBB, whereas adding a PGP inhibitor Reversan to the BBB led to higher cell death than without Reversan, indicating that Reversan aided the delivery of vincristine past the BBB to the brain to treat tumour cells.

With the advent of increasingly sophisticated organoids, there is growing demand for technology to replicate the interactions between organs. We anticipate that this platform can be utilised not only for testing BBB permeability of drug candidates and their effects on the brain, but also for studying the interactions between the BBB and the brain such as in the inflammatory response.

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A bioactive hybrid dECM-alginate system to unveil the role of the microenvironment in EMT/MET

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Microphysiological systems are rapidly emerging as a powerful tool to study human physiology and disease at cellular and molecular level. Here we develop a novel platform combining bioengineered peptide-alginate hydrogels with decellularized extracellular matrix (ECM) from human breast fibroblasts, to fabricate a biochemical and biomechanical relevant environment for the culture of epithelial cells, thus integrating the parenchymal and stromal compartments of breast tissue. We used the system to study the effect of ECM in cancer associated epithelial-to-mesenchymal transition (EMT) and its reversion (MET), using different techniques. Our results show that in RGD-alginate 3D matrices breast epithelial cells were able to grow and organize into acinar-like structures akin to the mammary gland, and that distinct EMT/MET states could be generated in the presence/absence of TGFb1, namely an epithelial-like state (E), two partial mesenchymal-like states (EM1 and EM2), and a reversed state from M to E (ME). As expected, compared to E, EM1 and EM1 cells showed decreased expression of E markers (OCLN, EPCAM) and increased expression of M markers (FN1,CDH2) and EMT transcription factors (SLUG, ZEB2). To improve the biomimicry of the model, we produced decellularized ECM under controlled *in vitro* conditions from TGFb1-activated fibroblasts (adECM), recreating a tumor-like matrisome that was solubilised and incorporated into alginate hydrogels. The dECM from normal (non-activated) fibroblasts was used as a control. Epithelial morphogenesis occurred in both groups, but the presence of adECM upregulated the expression of M markers compared to dECM. To analyse the complementary role of matrix

mechanical properties, the hybrid hydrogels (G´~200 Pa, typical of normal breast tissue) were stiffened by external ionic crosslinking (G´~2000 Pa, typical of breast tumors).Cells were able to mechano-sense matrix stiffness alterations, with upregulation of CYR61, CTGF, and ANKRD1, and nuclear YAP translocation. The presence of adECM and the increased stiffness synergistically induced EMT, as shown by the upregulation of prototypical markers. Collectively, our results illustrate the applicability our hybrid system as a versatile physiological 3D model to investigate the role of the ECM in EMT/MET.

Acknowledgments: REMODEL (857491-H2020-WIDESPREAD-03-2018). Portuguese FCT: project PTDC/BTM-ORG/5154/2020, fellowship SFRH/BD/31757/2017

References

[1] doi:10.1038/srep27072
[2] doi:10.3389/fbioe.2020.00494

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Toll-like receptors and organ-on-chip approaches for AD drug screening

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Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the formation of amyloid-β plaques and neurofibrillary tangles (NFT), which results in decreased synaptic transmission and cerebral volume loss. Recent studies in AD pathology emphasize the dual role of innate immunity during the progression of AD. Toll-like receptors (TLRs), an important family of pattern recognition receptors (PRRs) of the innate immune system, which produce the first line of defense against microbes, are found to be key mediators of many inflammatory processes and immune-related diseases. Notably, TLR2 and TLR4 have been proposed as promising targets for AD drug discovery and development. Several mediators with selective TLR2/4 agonist or antagonist activity have been developed and are likely to be beneficial to Alzheimer's patients. However, despite research efforts, AD continues to be one of the most susceptible disorders to drug development failure. The complexity of the human brain, particularly the difficulty of drug delivery across the blood-brain barrier, and the significant difference between animal models and humans are cited as the primary causes of the poor success rate in clinical trials. Recent advancements in micro-physiological systems (MPS), which also refer to organs-on-a-chip (OOC) models, have opened up new paths for AD research.

Aim: This study conducts a qualitative review of current microfluidic models for Alzheimer's disease drug screening by searching Scopus, PubMed, and Google Scholar for relevant review articles, research papers, and conference reports. We also discuss the limitations of existing AD-on-chips for drug screening and propose ways to improve the outcomes. This paper includes a list of TLR-targeting drugs that are currently in clinical trials as well. Based on the recommendations of researchers and experts in this field, we summarize the reasons for clinical trial failures and potential solutions to help further drug discovery and development in AD.

Conclusion: This study helps scientists identify the right directions and most promising tools for developing best-fit, rapid, high-throughput, cost-effective models for AD drug discovery, reducing clinical trial failures.

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Hyper-nutritional cell culture media distort the expression of anti-cancer drug targets

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Introduction: Nutrient availability in the tumor microenvironment has a crucial impact on cellular processes, metabolism and anti-cancer drug responses. However, the overwhelming majority of current *in vitro* biomedical studies use supra-physiological levels of nutrients in a non-flowing culture environment that distort the metabolism in cancer models and may undermine their predictive value. For example, paclitaxel as the most commonly used chemotherapy for lung cancer has a 25 to 500-fold discrepancy between its cell culture and clinically effective concentrations. We aim to better emulate the microenvironment to improve the predictive value of current cell culture microphysiological systems.

Methods: A medium with human plasma-like components and concentrations (Melbourne Medium, MM) was developed and supplied at a constant flow using the multiplexed superfusion system (*RPM2*) [1] to prevent nutrient depletion. We evaluated the phenotypic, proteomic and metabolomic impacts of MM and investigated the influence of MM on therapeutic target expression levels and drug responsiveness.

Results: Culturing A549 cells in MM compared to the conventional medium (CM) led to changes in cell morphology, proliferation and cell cycle distribution. Additionally, A549 cells displayed a more EMT-like phenotype with tight junction disruption and increased motility. We also observed a relative insensitivity to paclitaxel in MM compared to CM, accompanied by lower ß-tubulin abundance and fewer cells arrested in G2/M phase. Furthermore,

our proteomic and metabolomic analysis suggested that several key biological pathways involved in cancer progression were sensitive to hyper-nutritional (CM) media, including differential expression of several ß-tubulin isotypes. When deploying the *RPM2* system to facilitate the continuous MM supply, cell survival increased when compared to conventional static culture in CM. Cell viability was above 95% after 5 days perfusion of cultures with MM. Importantly, metabolomic profiling validated the culture system by showing maintenance of the physiological nature of the culture media, while simultaneously preventing the accumulation of certain bioactive metabolic end products.

Discussion: Our more physiologically relevant culture system provides a baseline with which to achieve a better emulation of the (patho)physiological microenvironment and its use may further improve the predictive value of cancer therapeutic screening.

Reference

[1] Gao, X. et al. (2022). *Lab Chip 22*, 1137-1148.

Presentation: Poster

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Complex model adoption at Genentech

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As MPS and complex models mature in their second decade since inception, it is important to explore how much of that important academic investment has translated to routine use in pharma. This talk will depart from the traditional methods-results-conclusions format and lean towards science strategy. The importance of the foundational data sets Genentech generates for organ systems and how we use that data to drive decision-making will be discussed. Additionally, while MPS use in regulatory filings is often debated in public settings, pharma's internal decision-making using MPS is equally, if not arguable, more important to demystify. The model requirements for the two scenarios will be covered at length. Furthermore, how decisions are made on throughput versus complexity will be underscored using several real-world examples. The talk will conclude with separate calls to action for the developer and pharma communities to continue to work in tandem to realize a future where more predictive tools are employed in the drug development process to improve human translational outcomes, all while reducing our collective reliance on animal models.

Presentation: Oral

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Human tendon-on-chip (hToC) platform for modeling fibrotic disease and screening therapeutic candidates

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Proliferation of myofibroblasts from quiescent tenocytes upon injury is influenced by the secretome of infiltrating immune cells and more specifically TGF-β1, leading to chronic inflammation and fibrosis. Yet the crosstalk between the tendon fibroblasts (TC), endothelial cells (ECs), and both resident and infiltrating immune cells has been understudied. Thus, we designed a modular human tendon-on-chip (hToC) to study cellular and molecular contributions of the complex microenvironment present in the healing tendon's fibrovascular scar. Consistent with *in vivo* tendon injury, we show that the addition of TGF- β1 results in myofibroblast differentiation, tissue contraction, senescent phenotypes, and activation of ECs consistent with inflammation in the hToC model.

The induction of fibrosis through treatment with exogenous TGF- β1 in this tendon-vascular interface model was similarly achieved by co-culturing with macrophages. Interestingly, the secretion of key Senescence Associated Secretory Phenotypes (SASPs) such as CXCL10, IL-1β, and TNF-α were drastically increased with the addition of monocytes. We found that these cells extravasate into the tendon compartment through a disrupted EC barrier and induce apoptosis/cell cycle arrest, activation of the mTOR pathway, and enhance cell proliferation, all of which are markers of fibrotic disease. With this model of peritendinous fibrosis, therapeutic doses of Ibuprofen and Rapamycin, selected in range of human serum levels during standard pharmacotherapy, were administered through the vascular compartment. Analysis of secreted cytokines and RNA sequencing of both compartments revealed that the chips modeled the donor-specific heterogeneity in SASP profiles and differentially expressed genes in response to either treatment.

Despite the proliferation of sophisticated MPS platforms to model various organs and tissues, including musculoskeletal tissues such as bone and cartilage, there has been minimal emphasis on modeling tendon pathobiology. The hToC, therefore, is a valuable new tool to understand the healing tendon and identify molecular targets for promoting scarless healing. Particularly the activation of the mTOR pathway and upregulation of key SASPs should allow for therapeutic screening of drugs that mitigate and resolve fibrotic pathology. Our data support the potential of the hToC as a patient-specific platform for precision medicine in the treatment of tendon injuries and other forms of fibrosis.

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A study on derivation of standardization items in organ-on-chip

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Organ on Chip (OoC) is a technology that emulates the mechanical and physiological cellular response of the human body as well as embodies the characteristics of the structure and function of a specific organ or tissue of the human body on a chip, which is an *in vitro* environment, and is used in various medical fields. Compared to animal models or 2D cell culture models, OoC can predict drug efficacy and toxicity with higher accuracy, so it is expected to be used as the most effective tool for developing new drugs and targeted therapies. Since the COVID-19 pandemic, organoid and OoC technologies have been attracting more attention as useful tools for studying viruses and developing vaccines and treatments, and can be used as tools to predict infectious diseases in unexpected pandemic situations in the future.

Standardization is needed for overall verification of OoC's structure, function, and specifications of performance requirements, and for safer and more efficient development and utilization of OoC. In this study, as the need for international standard development in the field of OoC emerges, recently published OoC-related papers are searched and OoC studies that are currently attracting attention and are being screened through the prisma 2020 tool. First, set the search period and keywords, and search PubMed with the following conditions.

Period: 2020.01 ~ 2023.01

After screening in the following steps, the Prisma 2020 screening results were analyzed in detail with a matrix table.

Step 1: Delete redundant papers

Step 2: Delete papers not related to OoC

Step 3: Select papers that include the need for standardization of OoC

In addition, global trends were identified by searching for reports and guidelines related to "OoC standardization". Through this study, the need for international standardization in the field of OoC is identified and the items that actually require standardization are confirmed to make the standardization items more concrete. (The results of the study will be included in the final poster.)

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Engineering patient-specific vascularized mini-brain-chips of immuno-glial-neurovascular units for accelerating drug development

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Up to 1 billion people worldwide, 1 in 6, are estimated to be afflicted with a neurological disorder, costing over $500B in the US. Treatments are hindered by the highly selective human blood brain barrier (BBB) and for many neurological diseases there is still no pharmacologic treatment that can slow or stop neuronal damage. A major bottleneck is that current models fail to recapitulate human brain tissue and BBB properties with sufficient fidelity. The rodent BBB is far less selective than human and does not mimic the complexity of human genetics. *In vitro* models have the advantage of enabling high throughput screening and real-time monitoring in human-based systems with tunable genetic backgrounds harnessing iPSC technology. The brain is comprised of 6 cell types arranged in stereotyped 3D architecture, each of which effects BBB properties. Microphysiological systems harnessing natural biomaterials have enabled the co-culture of 3 of the relevant cell types into reductionist BBB models. However, these lack the contributions of neurons, oligodendrocytes, and microglia, have not yet matched human brain BBB permeability and selectivity, and often use neurotoxic materials. To overcome these limitations we have engineered a soft hydrogel biomaterial with brain-specific biochemical cues that supports the self-assembly of all 6 brain cell types into a 3D multi-integrated brain (miBRAIN) immuno-glial-neurovascular unit-on-a-chip. This is the first brain tissue model to our knowledge that contains integrated microvascular and neuronal networks, mature neuronal markers, robust neuronal electrical activity and electrophysiological properties, transcriptional signatures closer to human brain tissue than their individual cell counterparts, mature BBB markers, and functional interactions between cell types. We have further harnessed our model to probe the effect of APOE4, the strongest genetic risk factor for sporadic Alzheimer's. We have engineered a novel microfluidic platform for 3D cell culture in engineered materials, which typically cannot be cultured in confined microfluidics due to their innate swelling properties. This 3D-miBRAIN-Chip model could be harnessed for high throughput therapeutic screening, real-time monitoring with high spatiotemporal resolution, probing disease mechanisms, and assessing pathological progression across patient-specific cohorts, all in an *in vitro* system with potential for expediting drug discovery and development.

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Development of an *in vitro* liver culture system for continuous bile recovery

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In the liver, blood and bile are the two excretion pathways for drugs metabolized by hepatocytes. Metabolites carried through blood are rapidly excreted through the urine while the ones excreted via bile are partly involved in enterohepatic circulation which makes the prediction of the excretion of metabolites out of the body complex. In addition, some drugs excreted via the bile can cause liver cholestasis by influencing the excretion of bile acids via the inhibition of transporters. For these reasons, understanding the excretion pathway of drugs, especially biliary clearance, is expected to be crucial in the development of new therapeutical targets.

In *in vitro* models, sandwich-cultured hepatocytes have been the golden standard for assessing biliary metabolites by allowing to physiologically reproduce the formation of bile canaliculi between adjacent hepatocytes (Xingrong Liu et al., 1999). However, since bile cannot be evacuated in such a culture system, bile has to be recovered from hepatocytes in an invasive manner, which causes the collected bile to be diluted excessively within the collection buffer, hampering an accurate measurement of biliary metabolites.

In this study, we aim at developing a novel *in vitro* hepatocyte culture system which enables continuous bile recovery by combining microfabrication techniques and coating via proteins related to bile canaliculi formation (Yue Zhang et. al., 2020). We fabricated a device that consists of a polydimethylsiloxane (PDMS) disk with radial microchannels (width: 3 µm, height: 5 µm) and a PDMS tube at its center, serving as a bile collection port connected with the microchannels. To confirm the transportation of bile from channels to the collection port, primary hepatocytes were exposed to fluorescein-labeled bile acid (CLF). CLF excreted by hepatocytes was then successfully recovered from the port. While promising results have been obtained in the present work, further studies, involving forced guidance of bile via coating with proteins involved in its transporter are expected to significantly improve the recovery rate and allow more accurate analysis of the bile.

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RealBrain® 3D neural micro-tissues: A high throughput platform for drug discovery in Alzheimer's disease and other neurodegenerative disorders

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Recent advances in 3D cell culture systems provide a more physiologically relevant alternative to current approaches for high throughput compound screening. RealBrain® 3D neural micro-tissues (Tessara Therapeutics) demonstrate several advantages over other 3D models – they are optically clear and they reach maturity within three weeks, during which time the human neuronal cells replace the initial synthetic biomaterial with a cell-secreted extracellular matrix. Robotic manufacturing has also been optimised to ensure reproducible and scalable production of RealBrain® micro-tissues. In addition to ARTIBrain™, our healthy RealBrain® model, we have now developed ADBrain™, a unique 3D human model of sporadic Alzheimer's disease that exhibits key pathological markers, namely, secretion of amyloid-beta peptides (Aβ), intraneuronal elevation of phosphorylated Tau (pTau) and neuronal loss.

We leveraged recent discoveries around the role of ferroptosis, a distinct form of iron-dependent programmed cell death that has been implicated as a mechanism of neurodegeneration in Alzheimer's disease, Parkinson's disease, and brain injury, to demonstrate the physiological relevance of our platform. Currently, the main tools for ferroptosis drug discovery are tumor or immortalized cell lines, which fail to capture the complex micro-environment of human neuronal tissue, a major contributing factor to the high failure rate of drug discovery. In the first demonstration of its kind, we utilized ARTIBrain™ and ADBrain™ 3D RealBrain® micro-tissues to create a human neuronal model of ferroptosis using validated compounds for the induction of this pathway (erastin and RSL3) and its rescue (liproxstatin and ferrostatin). Using a bespoke suite of assays that measure cell viability, cytotoxicity, protein expression, cell number and QC readouts we present two robust proof-of-concept 3D human neuronal models ideally suited to high throughput screening of drug candidates.

Multiple studies demonstrate that 3D RealBrain® micro-tissues are a breakthrough in organotypic neural cultures that will help drive innovation and knowledge translation. It will also significantly de-risk drug discovery pipelines by reducing cost and providing earlier insights into drug neurotoxicity and therapeutic efficacy. With current changes in the regulatory landscape, this work will also foster the adoption of non-animal-based screening approaches across the sector.

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Testing short-chain fatty acid effects on the efficacy of CAR T cells in a gut-on-chip system

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Pre-treatment of cancer patients prior to CAR T cell therapy significantly alters the level of short-chain fatty acids (SCFA) in the intestine, thereby potentially influencing the efficacy of the immune cell-based cancer therapy [1].

To investigate the underlying effects of SCFAs, we used the immunocompetent microphysiological Organ-on-Chip (OoC) system [2] of the gut expressing specific target proteins of CAR T cells. The results show that untreated CAR T cells cause a high degree of specific tissue damage.

However, pre-treatment of CAR T cells with SCFAs limits tissue damage in a dose-dependent manner. Among SCFAs tested, butyrate mediates the strongest protective effect. Tissue protective effects were recapitulated by immunofluorescence staining and the measurement of cytokines release and change in cell viability. These effects are mediated via histone deacetylases (HDAC) as inhibition of these enzymes decreases the SCFA effect.

We were able to show that SCFAs modulate CAR T cell activity in a dose-dependent manner via the HDAC pathway. Furthermore, we were able to establish an isogenic intestinal model based on human induced pluripotent stem cells to study the modulation of CAR T cell effects by SCFAs in a patient-specific model system enabling the identification of personalized biomarkers for cancer therapy efficiency.

References

[1] Luu, M. et al. (2021). Microbial short-chain fatty acids modulate CD8(+) T cell responses and improve adoptive immunotherapy for cancer. *Nat Commun 12*, 4077.

[2] Maurer, M. et al. (2019). A three-dimensional immunocompetent intestine-on-chip model as in vitro platform for functional and microbial interaction studies. *Biomaterials 220*, 119396.

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Construction of a blood-brain barrier (BBB)-on-chip model that can evaluate immune cell infiltration and barrier disruption

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The blood-brain barrier (BBB) limits the access of immune cells and mediators to the central nervous system (CNS) and maintains the immune privilege of the brain. BBB disruption has been identified in CNS disorders, such as multiple sclerosis. Assessment of BBB disruption and immune cell infiltration is important to understanding the pathophysiology of these conditions. To date, however, few *in vitro* models that can evaluate these factors using human cells have been reported. Here, we attempted to construct a human *in vitro* BBB model that can evaluate BBB disruption and immune cell infiltration using a microfluidic culture device, OrganoPlate® 3-Lane (MIMETAS B.V.). Immortalized human brain microvascular endothelial cells (TY10) were co-cultured with pericytes and astrocytes under perfusion stimulation, during which they formed a tube-like structure. Compared to the cell-free condition, this tube showed trans-endothelial electrical resistance (TEER) values of appropriately 8-18 $\Omega \times cm^2$ and inhibited extravascular leakage of 20 kDa FITC-dextran to under 45%. Administration of BBB disruption factors induced a decline in TEER value and increase in dextran leakage ratio from under 10% to more than 30%. These results suggest that the TY10 tube has endothelial barrier function. Perfused T lymphocytes infiltrated from the lumen across the TY10 endothelia to the outside and migrated in response to gradient CXCL12, a chemoattractant of T lymphocytes. Extravasation of T lymphocytes was increased when external CXCL12 concentration was increased. These results indicate that this BBB model would be useful in assessing barrier disruption and immune cell infiltration, which are part of the disease process of neuroinflammatory disorders. Moreover, this model can simultaneously test 40 chips/plate, and can be handled by an automation unit. Used in combination with an automation system, this model is expected to serve as a platform not only for understanding the pathophysiology of CNS disorders but also for drug discovery research.

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Application of human iPSC-derived 3D-structure alveolar organoids for translational IPF therapeutics research

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Therapeutics development for respiratory diseases have unfortunately been relatively unsuccessful, with its weaker success rate particularly in phase 2 proof-of-concept studies, compared to other disease areas [1]. This implies limitations in existing *in vitro* and *in vivo* models, and hence the need for a more predictive, human-relevant model that offers better translational value. We have been leveraging our human induced pluripotent stem cell (iPSC)-derived respiratory organoid models, based in part on cell differentiation protocols developed at Kyoto University [2], that reliably yield robust alveolar and airway epithelial cells. In particular, we have formulated 3D-structure alveolar organoids (3D-sAlvOs) that incorporate fibroblasts, thus reconstructing the complex interaction between the epithelium and the mesenchyme. We here report our application of 3D-sAlvOs to pulmonary fibrosis research, in which fibrotic agents conspicuously constrict the 3D-sAlvOs, and conversely, anti-fibrotic agents induce re-expansion of the constricted organoids. We leverage this "anti-fibrotic re-expansion assay", from which an index value is calculated from the ratio of the size of re-expanded organoid relative to its constricted size at baseline – after exposure to bleomycin as the fibrotic stimulus. We deployed this assay to nintedanib, a clinically approved drug for idiopathic pulmonary fibrosis (IPF) [3], along with a wide range of known IPF drug candidates, to compare anti-fibrotic strengths of each agent. We observed across these agents a correlational tendency between anti-fibrotic re-expansion index and successes in clinical trials. In addition, as the organoids can be readily subjected to single-cell sequencing and spatial transcriptomics, we have gained granular insights into how specific pathways, including abnormal tissue regeneration and senolysis, potentially play their roles in fibrotic pathophysiology and therapeutics. This predictive value, supported by both tissue-level objectivity and deeper high-content analyses, should greatly contribute to overcoming existing challenges in IPF therapeutics R&D, and to wider pulmonology research overall.

References

[1] BIO, QLS Advisors, and Informa UK. Clinical Development Success Rates and Contributing Factors 2011-2020. February, 2021.

[2] Yamamoto, Y. et al. (2017). *Nat Methods 14*, 1097-1106.

[3] Richeldi, L. et al. (2014). *N Engl J Med 370*, 2071-2082.

Presentation: Poster

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Investigating the anticancer potential of *Sutherlandia frutescens* in NHI-H69AR cancer mini-tumours

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As far as cancer related deaths is concerned, lung cancer is a major cause. Lung cancer can be divided into two broad histological subtypes, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). SCLC is known as the graveyard for drug development due to the failure of over 60 possible drug therapies. The development of drugs for the treatment of SCLC stayed stagnant for the last 30 years due to the rapid doubling time, high growth fraction and natural tendency to metastasize to the brain, liver, and bone early in the disease course. Most (70%) of the patients is diagnosed in the extensive stage of the disease due to its rapid growth and early widespread metastasis causing the overall survival rate to be just 6 months. The above-mentioned factors highlight the need for new drug development or alternative treatment methods.

During this study NHI-H69AR mini-tumour spheroids were cultured with the CelVivo™ Clinostar™ rotating bioreactor system. *Sutherlandia frutescens* (SF), an indigenous plant medicine with a long history of traditional use to treat cancer, was prepared as a water extract. This SF extract was applied to the spheroids at two concentrations, as well as in combination with the three chemotherapeutic drugs irinotecan, cisplatin and paclitaxel. The SF extract was able to reduce the spheroid planar surface area in a statically significant manner ($p < 0.05$) when compared to the untreated control. The soluble protein content as well as the intracellular adenosine triphosphate (ATP) values were statistically significant decreased following exposure to the higher SF dose when compared to the untreated control group. Furthermore, during the combination treatment studies it was observed that planar surface area, protein content as well as the intracellular ATP values were decreased in a scientifically significant manner.

SF alone and in combination with known chemotherapeutical drugs at clinical doses had a cytotoxic effect on NCI-H69AR mini-tumours, which shows promise for the phytomedicine to serve as a potential source for SCLC treatment.

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Implementation of an intestinal organ-model to investigate the human absorption and first-pass metabolism of pesticides

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In vivo rodent ADME studies are still mandatory to investigate the human safety of pesticides for regulatory purposes. However, new approach methodologies (NAMs) represent a future *in vitro* tool in the replacement, reduction and refinement (3R's) of animal studies [1] Within the framework of this approach, a static human intestinal organ-model with Caco2 cells is applied to investigate the intestinal absorption and first-pass metabolism towards the fungicide trifloxystrobin.

Immortalized human intestinal Caco2 cells were cultured on collagen I coated transwell inserts to form confluent and differentiated monolayers within 21 days. Barrier integrity of the cell monolayer was monitored by transepithelial electrical resistance measurement and lucifer yellow leakage into the basal compartment. Cell viability, differentiation and metabolic functionality was confirmed by determination of lactate dehydrogenase, alkaline phosphatase and glucose, respectively. On day 21, cells were exposed to 10 µM of a selected fungicide. Sampling of apical and basal specimens was performed after four hours of incubation. Subsequently, samples were semi-quantitatively analyzed by high resolution mass spectrometry and identified metabolites were compared to rat *in vivo* data.

Results revealed primarily the known phase I metabolite desmethyl-trifloxystrobin in the apical nutrition Medium [2]. Caco2 cells metabolized trifloxystrobin almost entirely during the 4 hours of incubation. The detection in the apical compartment indicated a low absorption and high metabolic capacity of Caco2 cells towards trifloxystrobin. Hence, building the assumption of increased fecal excretion of the fungicide and it's formed metabolite in human which also is in good agreement with the known rat *in vivo* data [2].

The static intestinal organ-model provides the opportunity to investigate the human absorption and first-pass metabolism of orally exposed pesticides. Predicting the exposition of pesticidal compounds towards downstream organs (e.g., liver) is crucial for human risk assessment. Moreover, the envisioned transfer into a dynamic chip technology provides the basis of future organ interactions in multi-organ chips and respective investigations.

References

[1] Directive 2010/63/EU of the European Parliament and of the council. *Official Journal of the European Union*.

[2] Peer review of the pesticide risk assessment of the active substance trifloxystrobin (2017). *EFSA J 15*, 4989.

Presentation: Poster

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Human 3D *in vitro* models for the assessment of cancer immunotherapy mode of action

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Cancer Immunotherapy (CIT) strategies have enabled significant breakthroughs in cancer treatment. However, their development remains extremely challenging, also due to intrinsic limitations of currently available preclinical experimental approaches. The next generation of CIT-drugs will require a more dynamic and content-rich analysis allowing for mid-throughput readouts, in the most physiological human tumor microenvironment (TME) possible.

In order to complement currently available 2D *in vitro* and *in vivo* preclinical experimental models, we utilized the microfluidics OrganoPlate 3-lane 40 system by MIMETAS, to establish two distinct, fully human, imaging approaches for the screening of next generation CIT-drug candidates.

The first system is a 3D *in vitro* imaging model which enables the dynamic visualization and characterization of interactions between cancer cells, immune cells, stromal cells, and extracellular matrix (ECM), recapitulating the human TME organization and immune cell dynamics. After carefully cross validating our platform against *in vivo* and 2D *in vitro* analyses, we now employ this new system to shed light on the Mode of Action (MoA) of immune cell engagers, such as Glofitamab.

The second system is a fully human mid-throughput 3D *in vitro* screening system for imaging and quantifying immune cell trafficking and infiltration to tumors in response to CIT-drugs. It combines endothelial tubules, peripheral immune cells, solid or liquid tumors, ECM and tumor-resident immune cells. This model can provide insights on the impact on immune cell infiltration of immunomodulation and T cell redirection CIT drug candidates.

As a next step towards more physiological 3D *in vitro* models, we aim at implementing Patient-derived Tumor Organoids (PDTOs) in order to allow for a more translatable assessment of the CIT-drugs MoA.

In summary, these models allow for mid-throughput screening of drug candidates, evaluation of combination strategies and a mechanistic understanding of their MoA in a fully human 3D *in vitro* system and are a valuable addition to existing preclinical models with their ability to mimic physiological immune cell dynamics, such as immunological synapse formation and trafficking. Consequently, these platforms support next generation CIT-drug development.

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Targeting respiratory viruses: A novel alveolus-on-chip infection model for pre-clinical applications

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Circulating respiratory viruses and the emergence of novel variants pose a constant threat to public health as highlighted by the pandemic outbreak of COVID-19. In the distal lung, SARS-CoV-2 infection interferes with normal function of type I and II alveolar epithelial cells (AEC), which results in the breakdown of the air-blood barrier and massive inflammation in severe cases. Due to the difficulty to model the alveoli, there is a lack of pre-clinical models to study the multifactorial pathophysiology of severe COVID-19 and test novel compounds in a human-relevant context. Human-derived microphysiological systems have the potential to contribute to a better understanding of the disease and speed up the development of targeted therapeutics.

In this work, we aimed to develop AlvireX, a drug screening platform for respiratory viruses. It combines alveolar microtissues on transwell inserts for medium-throughput screening and subsequent validation of shortlisted candidate drugs on a lung-on-chip (LOC) model.

We used an alveolar epithelial cell line (^{AX}iAEC) derived from primary human AEC and optimized for on-chip applications. ^{AX}iAEC cultures were infected with SARS-CoV-2 or influenza A virus (IAV) and monitored for virus production and barrier function by virus titration, immunofluorescence imaging and transepithelial electrical resistance (TEER) measurement.

^{AX}iAEC maintained an alveolar phenotype (HTII-280, ABCA3, SP-C, HTI-56) on transwell insert and LOC model. We found that essential host factors for SARS-CoV-2 cell entry (ACE2, TMPRSS2) were upregulated in air-liquid interface (ALI). Consequently, ^{AX}iAEC were susceptible to SARS-CoV-2 infection in ALI culture and released viral progeny. TEER significantly decreased in infected cultures indicating virus-mediated barrier breakdown. Finally, infection under physiological stretch resulted in more efficient SARS-CoV-2 production than in static conditions. Additionally, we demonstrated productive IAV infection in ^{AX}iAEC resulting in the release of high viral titers. In comparison to SARS-CoV-2, IAV replicated more rapidly and efficiently, which reflects their differential infection dynamics in the alveoli.

Our results highlight the importance of a complex microenvironment (ALI, biomechanical cues) to model viral infection and immune response in the alveoli. Being susceptible to SARS-CoV-2 and IAV, we believe that the AlvireX platform will be broadly applicable to target current and emerging respiratory viruses.

Presentation: Oral

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Characterization and robust cultivation of porcine intestine stem cells toward animal testing replacement

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Intestine stem cells and three-dimensional (3D) organoid technology offer great promise for disease modeling based host-pathogen interactions, pesticide toxicity assessment and feed efficiency measurement in farm animals as well as regenerative medicine for therapeutic purposes in humans. However, characterization and long-term cultivation of intestine stem cells in porcine require further investigation in details. Recently, significant efforts have been made to establish an *in vitro* system for 3D organoid culture of porcine intestine stem cells for animal testing replacement and practical application including stem cell therapy and translational research. In this study, porcine intestine stem cells from different site of small intestine in weaning piglet and adult pig were characterized with several specific markers *in vivo*, and sequentially isolated. Furthermore, intestine stem cells were long-term cultivated and evaluated the expression of several specific markers representing crypt-villus structure. In addition, they showed the key functionality with regard to paracellular permeability was maintained during several passages of culture. Collectively, these results indicate the efficient cultivation and characterization of porcine intestine stem cells based on 3D organoid cultivation. Finally, these porcine intestinal organoids will facilitate the potential use for various purposes in the field of microphysiological systems based on animal physiology.

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Proteomic-phenotypic dual profiling on anti-angiogenic drugs using high-throughput microfluidic platform

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Angiogenesis plays a critical role in pathological development and is considered as a major breakthrough to overcome diseases [1]. However, only a few anti-angiogenic drugs are available while a large number of drugs have been developed for decades [2]. Despite advances in *in vitro* platforms to screen various drugs under numerous conditions, two-dimensional(2D) assays are still widely used and showed results discrepant from *in vivo* environments raising the need for 3D models. Here, we present a 3D high-throughput microfluidic platform that enables the evaluation of anti-angiogenic drug performance. This platform is manufactured by injection molding using polystyrene and consists of 16 wells allowing reproducible reconstruction of vascular structures. The robust formation of endothelial monolayer during the early stage of our culture system enabled the uniform reconstruction of fibroblast-stimulated angiogenic sprouts. After the treatment of angiogenic inhibitors (bevacizumab, cabozantinib, and paclitaxel), dose-dependent responses were effectively demonstrated via phenotypic quantification of sprouts from fluorescence images and protein expression change analysis by mass spectrometry. The results indicate that this *in vitro* platform is suitable for representing 3D angiogenesis followed by an in-depth multifaceted analysis of drug efficacy and is expected to contribute to high-throughput drug development.

References

[1] Carmeliet, P. (2005). Angiogenesis in life, disease and medicine. *Nature 438*, 932-936.

[2] Jung, S. Jo, H. Hyung, S. et al. (2022). Advances in 3D vascularized tumor-on-a-chip technology. In *Microfluidics and Biosensors in Cancer Research: Applications in Cancer Modeling and Theranostics* (231-256). Cham: Springer International Publishing.

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Vascular inflammation modulates trans-endothelial electrical resistance and immune cell migration in a scalable organ-on-a-chip platform

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The vasculature system plays a critical role in the body inflammation processes. Vascular inflammatory mechanisms are characterized by disruption of blood vessel wall permeability together with increased immune cell recruitment and migration. There is a critical need to develop models that fully recapitulate changes in vascular barrier permeability in response to inflammatory conditions. To this extent, we demonstrate a platform for inducing inflammatory reaction in HUVEC based microfluidic barrier models combined with continuous Trans Epithelial Electrical Measurements and coculture with Human peripheral Blood mononuclear cells. We found that the endothelial barrier changes differently depending on the inflammatory cytokine or immune cells added leading to changes in ICAM expression and endothelial morphology. We propose our platform as an essential tool for modeling endothelial inflammation in combination with immune cell interaction that can be used to screen for different targets and drugs to treat chronic vascular inflammation.

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Pump-less organ-on-a-chip platform: A versatile tool for cell biological research

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Organ-on-a-Chip (OoC) systems are a key technology of the 21st century. Most academic and commercial systems either use internal or external pumps for fluid actuation, which are prone to malfunction or suffer from air bubbles. This, in turn, reduces long-term stability and reproducibility [1]. Pump-less platforms are considered more user-friendly, robust and scalable, and can be a game-changer to build-up more predictive and complex human-like organ models for substance testing or disease modeling.

We developed a pump-less recirculation OoC (rOoC) platform that generates a directional, gravity-driven flow in two perfusion channels separated by an extracellular matrix barrier encompassing two 3D culture chambers [2]. The platform allows endothelial lining, blood vessel sprouting, the (re-)circulation of cells, and the incorporation of 3D cell models. Moreover, we present a computational model to predict the wall shear stress (WSS) and mass transport on the rOoC platform.

A current limitation is that the flow is pulsatile. Therefore, we introduce a novel perfusion system capable to control the WSS in a wider range. Next, we show proof-of-concept on a drug-induced liver-injury model that recapitulates the complex interactome between resident human stem cell-derived liver organoids (HLO) and circulating immune cells. 3D-HLOs could be cultured for at least 2 weeks in the rOoC without a significant decrease in viability, albumin and urea production levels [2]. Peripheral blood mononuclear cells (PBMCs) stayed vital "on chip" for over 24 h and were transported in the fluidic channels without activation [2]. After treating the HLOs with acetaminophen (APAP), an increased migration of lymphocytes towards the APAP-treated HLOs could be observed. To study this migration process, a novel online-monitoring device is introduced that is compatible with the rOoC platform and which is capable for parallel observation of several rOoCs.

In summary, the presented rOoC platform offers a convenient novel tool for disease modelling including models that require interactions between organ representations and components of the immune system in a robust, reproducible and scalable way.

References

[1] Busek, M., Aizenshtadt, A., Amirola-Martinez, M. et al. (2022). *Biosensors 12*, 126.

[2] Busek, M. et al. (2022). *Lab Chip*. doi:10.1039/D2LC00919F

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Immune cell extravasation in lung infection – to tilt or not to tilt?

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The acute respiratory distress syndrome (ARDS) is a severe lung condition with high mortality hallmarked by hypoxia, severe lung damage and inflammation. One of the main causes of ARDS is infection, for which no good treatment is available. Hence, more research into the pathophysiology of ARDS is needed. Since classic cell culture models lack the complexity of an immunocompetent lung and the immune system of model animals is significantly different, new model systems are needed. Infection within existing lung-on-chips lacks a natural protein membrane barrier. Here, immune cell extravasation into a hydrogel barrier upon infection was investigated.

A three-channel lung-on-chip was designed and produced by PDMS soft lithography. The central channel contains a hydrogel lined on one side by an endothelial barrier and by an epithelial barrier on the other side. Hydrogel protein concentration was varied, and shear stress can be applied with a pump or on a tilting platform. A chemotactic gradient was established across the hydrogel barrier and immune cells were perfused through the endothelial channel to observe their migration.

First, migration of immune cells across an endothelial barrier into a hydrogel barrier was investigated. Here, it was shown that extravasation depends on hydrogel density, with less extravasation

into higher density hydrogels. Moreover, when perfusing flowing immune cells and chemoattractant with a pump, creating a constant unidirectional flow pattern, extravasation of immune cells occurred earlier and more cells extravasated than when the assay was carried out on a tilting platform, exposing the cells to bidirectional, non-continuous flow. Lastly, lung endothelial and epithelial cells were cocultured and immune cell migration upon lung infection was quantified, showing increased immune cell migration in the presence of an epithelium.

A perfusable lung infection-on-chip was developed. This study underlines the impact of ECM density and flow profile on immune cell extravasation upon infection. Next, the model can be used for the study of immune cell migration in ARDS under different conditions, such as ECM composition and density, exposure to different infectious agents and presence of different cell types.

This project has received funding from the EU Horizon 2020 programme under grant agreement [812954].

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Towards a fully automated drug testing platform with engineered neural networks *in vitro*

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Drug screening for neurological diseases is still a challenging task. While *in vivo* studies in animal models come with considerable ethical concerns and suffer from limited transferability to humans, *in vitro* networks underly drastic variability depending on the culturing conditions, network connectivity and cell source.

In bottom-up neuroscience, small neural networks of reduced complexity are engineered by seeding primary rat neurons or human induced pluripotent stem cell (hiPSC) into polydimethylsiloxane (PDMS) microstructures on top of microelectrode arrays (MEAs) to spatially confine the location of their soma and guide neurite growth [1]. Repetitive stimulation of these networks yields stable and reproducible spiking patterns in the evoked responses over several hours [2], which can be modulated by adding drugs [3].

In this work we present a custom readout and incubation system, *Inkube*, with recording and stimulation capability for 4 standard 60-electrode MEAs in parallel as well as fully integrated temperature and CO_2 control. Additionally, a closed-loop syringe pump based perfusion system with feedback on the fluid level in the wells is included. This enables the isolation of drug induced effects from variations caused by fluctuating pH and osmolarity changes and minimise perturbation during medium exchange. The highly modular system can be adapted to accommodate a wide range of experimental needs. All electronics utilise off-the-shelf components. A field-programmable gate array (FPGA) is programmed for data readout and interfaced with a python software for effortless experiment design. We demonstrate closed-loop adaptation of stimulation patterns depending on the received spike response.

With this approach we aim to increase reproducibility in network studies and provide a robust drug screening tool that serves the 3Rs principle (replacement, reduction, refinement).

References

[1] Girardin et al. (2022) *Lab Chip 22*, 1386-1403.
[2] Ihle et al. (2021) *Biosens Bioelectron 201*, 113896.
[3] Girardin et al. (2022). *bioRxiv* 2022.11.07.515431.

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Fabrication and development of emulsion-templated porous materials for 3D cell culture

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Microphysiological systems (MPS) have been developed to study the primary function of human organs *in vitro*. This study aims to evaluate the application of emulsion-templated porous biomaterials in MPS. Porous biomaterials are one of the most known and attractive materials in studying *in vitro* models. A suitable 3D bio-scaffolds known to be used for tissue regeneration as 3D substrate for better cell attachment, nutrition flow, infiltration and cell migration within the host tissue [1]. 3D structures with pore sizes at the range of 100-500 µm (macropores) provides good interconnectivity to allow cell attachment on the surface, tissue ingrowth and vascularization [2]. It was also aimed to fabricate and develop highly porous biomaterials in macro sizes from the high internal phase emulsions (HIPEs) consisting of more than 75% of the volume, hence such materials have great demands in the field of MPS and regenerative medicine. A combination of macroporous emulsion-templated biomaterials with MPS technology have potential to facilitate mass and oxygen transfer into the tissues and enable a novel 3D platform for various cell types and vascularization compared to biomaterials with pore sizes < 100 µm. Thus, there is demand for versatile material with flexible porous structures, mechanical and chemical characteristics. The current state of the project showed that the fabricated biomaterial indicating good morphology and characteristics which makes it feasible for its ap-

plication in MPS. The rheological properties of the material under study were evaluated and the fabricated scaffolds were characterized by micro-CT and SEM. The pore size observed around 250-350 µm that support the application of this material as a 3D environment for *in-vitro* studies.

References

[1] Bokhari, M., Carnachan, R. J., Przyborski, S. A. et al. (2007). Emulsion-templated porous polymers as scaffolds for three dimensional cell culture: Effect of synthesis parameters on scaffold formation and homogeneity. *J Mater Chem 17*, 4088-4094. doi:10.1039/b707499a

[2] Maksoud, F. J., Velázquez de la Paz, M. F., Hann, A. J. et al. (2022). Porous biomaterials for tissue engineering: A review. *J Mater Chem B*, 8111-8165. doi:10.1039/d1tb02628c

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Investigation of the effects of blood degradation products on brain endothelium and astrocytes by an *in vitro* BBB model

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Subarachnoid hemorrhage (SAH) is a critical hemorrhagic stroke with poor outcomes. Blood brain barrier (BBB) dysfunction plays a crucial role in the pathophysiology of SAH [1]. Post SAH, blood components, and their degradation products activate many inflammatory and other metabolic cascades causing BBB dysfunction [2]. A better understanding of how blood degradation products affect the BBB cells could provide us with better therapeutic targets for the SAH treatment. However, traditional culture methods lack complexity and cellular responses cannot be recapitulated fully. The use of three-dimensional *in vitro* models and microfluidic platforms may overcome many of those disadvantages [3]. This study aims to develop an *in vitro* BBB platform to investigate the cellular response of brain endothelium and astrocytes against blood degradation products due to SAH. In transwells, we co-cultured primary human astrocytes and primary brain micro endothelial cells. The "brain side" was exposed to hemoglobin (100 µM). After five days, a transcriptome evaluation of both cells was performed. We found that hemoglobin-exposed astrocytes presented higher transcription levels of prostaglandin-endoperoxide synthase 2 (also known as Ciclo-oxygenase-2), interleukin 1a and 1b, as well as the interleukin 1 receptor. In the endothelial side, we found downregulation of adhesion cells such as claudin 1 and E-selectin in the hemoglobin-exposed group. Further analysis of transcriptome data might allow us to better understand astrocyte and brain micro endothelial cells response to hemoglobin and the identification of therapeutic targets. Adaption of the organ-on-a-chip technique will empower the investigation of human BBB in both preclinical and clinical studies. Thus, at the end of this project this platform will be ready to be used for disease modelling and drug screening studies.

References

[1] Kanamaru, H. and Suzuki, H. (2019). Potential therapeutic molecular targets for blood- brain barrier disruption after subarachnoid hemorrhage. *Neural Regen Res 14*, 1138-1143.

[2] Tso, M. K. et al. (2021). Gene expression profiling of brain endothelial cells after experimental subarachnoid haemorrhage. *Sci Rep 11*, 7818-7818.

[3] Vivas, A. et al. (2022). Aneurysm-on-a-chip: Setting flow parameters for microfluidic endothelial cultures based on computational fluid dynamics modeling of intracranial aneurysms. *Brain Sci 12*, 603.

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Assessment of vasculogenic potency of bone marrow derived stem/ stromal cells from multiple myeloma and acute myeloid leukemia patients in a microfluidic chip

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Acute myeloid leukemia (AML) and multiple myeloma (MM) are hematological malignancies that develop in the bone marrow.

Mesenchymal stem/stromal cells (MSCs) are an essential component of the bone marrow as they are a source of perivascular cells [1]. Recently, it has been acknowledged that bone marrow derived MSCs (BMSCs) from MM and AML patients are functionally impaired and may convert into active contributors to disease progression and therapy resistance [2,3]. In this study, we assess the vasculogenic potency of BMSCs from AML and MM patients compared to BMSCs from healthy donors.

Bone marrow aspirates were obtained from AML and MM patients and healthy donors with the donor's informed consent. BMSCs were isolated as the adherent cell fraction after culture of the mononuclear cells. BMSCs are characterized by analyzing surface marker expression using flow cytometry and by assessing their differentiation capacity towards osteogenic, adipogenic and chondrogenic lineages.

Microvascular networks are formed *in vitro* by embedding human umbilical vein endothelial cells (HUVECs) and BMSCs in fibrin gel and injection into AIM Biotech microfluidic chips as previously described [4]. A three-dimensional vasculature was formed during 6 days of co-culture in endothelial growth medium. The morphology of the networks is assessed after immunostaining for endothelial marker CD31 and pericyte marker alpha smooth muscle actin. Confocal microscopy and image analysis are performed to measure length of the network, average vessel diameter, and pericyte coverage of the network. Fluorescent microbeads will be introduced to the chips to assess the perfusability of the microvascular networks.

Our preliminary data suggest that microvascular networks formed in the presence of BMSCs from AML and MM patients are morphologically distinct from networks formed with healthy donor BMSCs. In the future, the patient-specific microvascular networks will be combined with the patient's malignant cells to assess the drug response of malignant cells and the bone marrow vasculature.

References

[1] Sweeney and Foldes (2018). *Front Cardiovasc Med 5*, 154. doi:10.3389/fcvm.2018.00154
[2] Diaz de la Guardia et al. (2017). *Stem Cell Rep 8*, 1573-1586, 2017. doi:10.1016/j.stemcr.2017.04.019
[3] Maiso et al. (2021). *Cancers 13*, 2542. doi:10.3390/cancers13112542
[4] Mykuliak et al. (2022). *Front Bioeng Biotechnol 10*, 764237. doi:10.3389/fbioe.2022.764237

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Encapsulating micro-engineered heart tissues-on-chip in bioactive hydrogels to integrate vascularization

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Since the advent of the first organs-on-chips, integration of perfusable vasculature has been achieved by e.g. engineered microfluidic channels, micropatterned gels or self-organized endothelial cell networks. However, integrating perfusable vasculature throughout high-density, 3D tissues in organ-on-chip systems is still a major challenge. Here, we are working on tackling this challenge by embedding 3D engineered microtissues a bioactive hydrogel.

We are using a three-lane chip, where the central hydrogel channel is flanked by two medium channels, and separated by phaseguides. Initially, a micro-engineered heart tissue (μEHT) is created in the central channel, by injecting iPSC-derived cardiomyocytes and adult human cardiac fibroblasts together with a mix of Matrigel and fibrin to provide extracellular matrix proteins. This cell-gel mix will compact over the course of three days, and form a μEHT around two flexible PDMS pillars centered in the hydrogel channel. After the formation of the μEHT, the central channel is coated with polydopamine, and a fibrin hydrogel is injected in the central channel, loaded with iPSC derived endothelial cells (iPSC-ECs). By substituting the conventional Tris-HCl buffer for polydopamine coatings with cardiomyocyte culture media, the central hydrogel chamber can be coated with polydopamine while a living tissue is present. This allows for the attachment of the fibrin based hydrogel to the PDMS walls of the chip, without interfering with the viability of the μEHT.

The seeded hydrogel remained stable up to 14 days in culture, and no degradation of the hydrogel was reported despite the continuous contractions of the cardiac tissue in the system. Additionally, the fibroblasts present in the μEHT start sprouting into the hydrogel after 7 days. When iPSC-ECs are present in the hydrogel, they are able to form a vascular network, which connects to both medium channels and the μEHT.

Our approach demonstrates that self-assembled vascular networks can be formed around preformed 3D microtissues using bioactive hydrogels. In ongoing work, we are further optimizing the formation of the vascular network through the addition of VEGF growth factor gradients, and the addition of hydrostatic flow through the hydrogel.

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Emulating physical dynamicity of arterial blood vessels and neighbouring tissue interaction

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Vasodilation and vasoconstriction are inherent to the circulatory system as part of biological response to various stimuli [1]. While the engineering of multiscale, branched, and interconnected blood vessels has been a well-investigated challenge, mimicking the dynamic (e.g., dilating – deflating) behaviour of native arterial vessels has remained understudied. In addition, reproducing the diffusion in pulsatile flow as well as the mechanical actuation transmitted by blood vessels to neighbouring cells and tissues remains an unexplored area of research in the organ-on-chip (OoC) field.

Here, we utilized a highly elastic hydrogel composed of tyramine-conjugated alginate (A-TA) to enable controlled and reversible dilation during steady as well as pulsatile flow. We utilized the novel ATPS (Aqueous two-phase system) bioprinting approach to 3D print branching vessel networks within living matter to gain control over the flow behaviour within living tissue models [2]. The influence of pulse frequency on diffusion as well as dilation was investigated. This novel approach offers improved supply nutrition, which can prevent the formation of necrotic cores in larger organotypic cultures. Moreover, it represents an actuation approach that mimics native mechanical stresses caused by pulsatile blood flow [3,4]. Thus enhancing differentiation and functionalization as a response to mechanical and biochemical cues. The flow related shear stresses are monitored by flow modelling to investigate the influence of the dilation onto the maximum shear within the channels. Further, local stiffening (limiting locally the dilation) can be a potential tool to model disease phenomena seen in stenosis or aneurism.

The next step of this study is to integrate such dynamic biomaterial into an OoC platform for added complexity and dynamicity by diffusing nutrients and mechanically to cyclically actuate the model by emulating the effects of the arterial blood flow.

References

[1] Fung, Y.-C. (1993). *Biomechanics: Mechanical Properties of Living Tissues*. Springer.

[2] Becker, M. et al. (2022) Aqueous two-phase enabled low viscosity 3D (LoV3D) bioprinting of living matter. *Adv Sci*, 2204609.

[3] Park, D. et al. (2020). Integrating organs-on-chips: Multiplexing, scaling, vascularization, and innervation. *Trends Biotechnol 38*, 99-112.

[4] Rouwkema, J. et al. (2008). Vascularization in tissue engineering. *Trends Biotechnol 26*, 434-441.

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Optimization of iPSC-derived endothelial cell culture in 3D hydrogel-based vessel-on-chips for studying long-term vascular dysfunction

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Three-dimensional hydrogel-based vessel-on-chips (VoCs) integrate physiological geometry, extracellular matrix (ECM) proteins, matrix stiffness and shear stress and have been proven to successfully mimic vascular (dys)function. Use of human induced pluripotent stem cell (hiPSC)-derived vascular cells in these VoCs is advantageous compared to their primary counterparts since they offer a robust source of (patient-specific) cells and allow for studying the effect of genetic background. Various strategies regarding medium formulations and ECM composition have already been reported for hiPSC-derived endothelial cell (hiPSC-EC) culture in collagen type-1 VoCs showing functional monolayers for 1-3 days. However, prolongation of cell culture time is required when studying endothelial dysfunction that occurs on longer time scales, such as endothelial-to-mesenchymal transition observed in atherosclerosis and pulmonary arterial hypertension.

We compared stability of iPSC-EC monolayers in viscous finger patterned collagen type-1 VoCs over time by assessing endothelial marker expression and barrier function through fluorescence angiography. Different medium formulations and additives as well as ECM compositions were tested. Endothelial cell growth medium ECGM2 and differentiation medium [1] completed with 1% penicillin/streptomycin were compared in absence and presence of ROCK-inhibitor. ECM compositions tested were various collagen concentrations, basement membrane protein coatings and additional crosslinking using genipin.

Fluorescence angiography 48 h after cell seeding revealed stable hiPSC-EC monolayers with an average permeability of $2.2 \cdot 10^{-5}$ cm/s in genipin-crosslinked collagen VoCs using EMG2, whereas the shear stress caused by the angiography damaged monolayers irreparably in other conditions. This indicates that monolayer stability is accelerated by crosslinking with 10 mM genipin for one hour as an additional fabrication step prior to cell seeding. Permeability values remain stable after the next 7 days. Also, VE-cadherin expression indicates an endothelial phenotype for prolonged microfluidic cell culture.

In summary, hiPSC-ECs cultured in genipin-crosslinked collagen type-1 VoCs using EGM2 medium produce stable monolayers suitable for assessing endothelial permeability after 48 h and stable for in total at least 9 days. We plan to study the long-term

effects of cytokine-stimulated endothelial-to-mesenchymal transition.

This project is part of the PHAEDRA-IMPACT consortium funded by the Netherlands Heart Foundation.

Reference

[1] Orlova, V. V. et al. (2014). *Nat Prot 9*, 1514-1531. doi:10.1038/nprot.2014.102

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Integration of patient-derived microtumors and autologous immune cells into tailored organ-on-chip platforms for the study of cancer-immune interactions and pharmacological interventions

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The costs of developing novel and more effective anti-cancer drugs have risen steadily over the last years partly due to the limited translatability of currently applied preclinical research models. In this regard, organ-on-chip platforms hold promise to better recapitulate the *in vivo* situation of human diseases and hence to improve pre-clinical prediction of drug efficacy.

Ovarian cancer (OvCa) is one of the deadliest cancer types in women mostly due to the lack of early prediction markers and non-specific symptoms. Additionally, OvCa is characterized by a high degree of intra- and intertumoral heterogeneity, which significantly impacts individualized treatment response. Supported by Wellcome LEAP as part of the HOPE program, OvCa patient-derived microtumors (PDMs) are integrated into tailored organ-on-chip platforms to provide a patient-centered model system with improved insight into disease biology and treatment response.

Protocols for long-term (14 day) on-chip culture with sustained cell viability have been successfully developed for n = 7 ovarian cancer PDM models to date. PDM viability together with expression of ovarian cancer related markers (incl. EpCam, p16, Rb, p53, WT1) is monitored by live cell and immunofluorescence staining in parallel with qRT-PCR analyses. Moreover, on-chip co-culture of autologous immune cells with OvCa PDMs enables the quantification of inflammatory and cytotoxic immune cell responses using bead-based immunoassay analyses of perfused media over time.

In the further course of the project, established read-out technologies will be applied to OvCa-PDM immune cell co-culture exposed to conventional as well as experimental chemo- and immunotherapeutic mono- and combination treatments to quantify long-term drug efficacy in parallel to immune cell responses.

Finally, this PDM-on-chip model will be integrated into a multi-organ-chip to enable the analysis of crosstalk between perfused patient-derived tumor and immune cell compartments over time in the presence and absence of drug treatment.

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Microfluidic perfusion system for single organoid culture and isolation of its secreted extracellular vesicles

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Extracellular vesicles (EVs), a pathway of intercellular communication, are an emerging class of biomarkers for studying wide range of pathophysiological processes. So far they are mainly investigated from 2D cell culture medium or biological fluid samples, which are partially representing *in vivo* extracellular matrix microenvironment or cellular stretches and interactions [1]. Scientific community is gradually beginning to take interest in EVs secreted by 3D culture recognized as more relevant physiological models. Separating EVs from complex media in a specific and selective way is still challenging. Microfluidic devices are promising tools as they provide high throughput and automation and require low sample volumes. Here we explore new methods for the EVs capture from organoids secretions.

We develop microfluidic methods enabling the hydrodynamic trapping principle of a single organoid onto a microfluidic cartridge [2]. Our automated microfluidic device maintains constant perfusion of culture medium as well as constant collection of secretions. The developed architecture allows the integration of micro-pumping functions for a local and precise control of small volume of organoid secretions. Our microfluidic cartridge is composed of both a pneumatic and a fluidic layer which enclose a hyper-elastic membrane. In the pneumatic layer, channels are mi-

cromachined and connected to a pressure source to push the membrane towards the fluidic channels, thus creating a valve array for the fluidic layer

This integrated bioreactor platform is to be coupled with methods enabling the downstream isolation of EVs from whole organoid secretions using integrated and non-integrated approaches such as filtration, immunomagnetic capture, ultracentrifugation and size-exclusion chromatography. These orthogonal methods are compared in this work to determine which is the most appropriate one, or combination of those ones for isolation of EVs. Standard biophysical characterizations such as Nanoparticle Tracking Analysis (NTA) or ELISA assay of the isolated EVs are carried out to assess the efficiency and purity of the capture.

This work is paving the way for a full on-chip integration and for an integrated companion test assay based on EVs secretion tracking from multi-organoids.

References
[1] Gunti, S. et al. (2021). *Cancers*, 2072-6694.
[2] Quintard, C. et al. (2022). *Biosens Bioelectron*, 0956-5663.

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In vitro axon structuring in microfluidic devices with nanoscale spatial constraints

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The biochemical and physical mechanisms behind neuronal cell maturation and morphology, neurite development, specifically axonal growth cone formation and its exploration of the environment remain only partially investigated. Studying the impact of these critical factors *in vivo* is difficult due to the complexity of the extracellular environment. At the same time, observing these phenomena *in vitro* offers in-depth analysis and greater reproducibility. *In vitro* platforms allow for control over extracellular content, directionality, stiffness and porosity of the substrate material [1], all of which have certain influence on cell maturation and neurite development. However, these mechanisms differ substantially between *in vitro* and in vivo neuronal systems [2]. For example, neurons in the brain are surrounded by the soft extracellular matrix while most of the common *in vitro* settings involve culturing cells on hard glass substrates. Additionally, it is unclear how the morphology varies between neuronal cell types. It is important to recognize and characterize these differences to be able to translate insights gained from *in vitro* experiments to actual neurological systems. Recently, the development of neurofluidic systems for modeling neurodegenerative disorders [1] or drug testing has been growing in popularity. One aspect that many of these systems typically do not take into account is how the axon-microchannel interaction changes the overall dynamics of the axon. In this work, we study axonal growth dynamics using various types of rat primary neurons cultured in custom-designed polydimethylsiloxane (PDMS) microfluidic devices containing nanochannels [3]. The microstructure design allows for separation of cell soma from axons and allows for studying axons in detail. Varying the nanochannel size, we show the spatial limitations for axonal growth. Furthermore, we analyze the formation and structuring of axonal bundles, their dependence on cell density and on the type of spatial constraint as well as how these aspects differ between neuronal cell types.

References
[1] Osaki, T. et al. (2018). *Adv Health Mat*.
[2] Polleux, F. et al. (2010). *CSH Perspect Biol*.
[3] Mateus, J. C. et al. (2022). *ACS Nano*.

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In vitro mechanical and electrical mapping of hPSC-derived cardiomyocytes

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The contraction of cardiomyocytes (CMs) is a fascinating biochemical reaction in which the flow of ions is transformed by cells into mechanical contractions, representing the physiological state of the CMs. The dysregulation of this process is closely related to the fundamentals of cardiac diseases, making it critical to quantitatively measure contractile activity for better understanding and characterization of CMs contraction. Isolated CMs from adult rodents represent the gold standard for *in vitro* models, but they quickly degrade after a few hours, limiting the time window of the experiments. On the other hand, human pluripotent stem cells (hPSCs) offer a valid alternative source for studying cardiac cell biology, drug development, and cardiac therapies with the advantage of long-term culture capability. We are employing several techniques to monitor the activity of CMs in terms of electrochemical reaction and mechanical contraction. By combining FluidFM [1] and Traction Force Microscopy (TFM) [2], we can obtain a time resolved force map of the contraction of CMs with nano-Newton sensitivity in both apical and basal cell side, across the vertical and horizontal direction. Additionally, FluidFM enables the application

of mechanical or electrical stimuli. High-resolution CMOS-based microelectrode arrays (MEAs) are used to record the spontaneous electrical activity of CMs [3]. With these state-of-the-art techniques combined, we are able to generate CMs contraction activity propagation mechanically and electrically, revealing the cluster automaticity of hPSCs-CMs.

After mapping the in-plane and out-of-plane contraction forces and electrical activity of CMs, we focus on comparing CMs activity by inducing either an extracellular stimulus from CMOS MEAs or applying force-controlled drug delivery by FluidFM.

References

[1] Meister, A., Gabi, M., Behr, P. et al. (2009). FluidFM: Combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond. *Nano Lett 9*, 2501-2507.

[2] Bergert, M., Lendenmann, T., Zündel, M. et al. (2016). Confocal reference free traction force microscopy. *Nat Commun 7*, 12814.

[3] Müller et al. (2015). High-resolution CMOS MEA platform to study neurons at subcellular, cellular, and network levels. *Lab Chip 5*, 2767-2780.

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Microvessel-on-a-chip model for studying ultrasound and microbubble-mediated drug delivery

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The blood vessel wall creates a barrier which can impair the transport of drugs from the blood to the underlying tissue. Lipid-coated gas microbubbles (diameter 1-10 µm) oscillate upon ultrasound application which can be used to locally enhance vascular permeability. However, the mechanism underlying this effect is poorly understood. Furthermore, it is yet to be discovered what ultrasound settings maximize the treatment outcome. This study aimed to create a microvessel-on-a-chip model to investigate the effects of ultrasound and microbubble treatment on vessel permeability and cell viability.

Human microvascular endothelial cells were seeded against an extracellular matrix gel in the Organoplate® 3-lane and cultured for 4 days under bidirectional flow to form a 3D microvascular tube (300×220×2200 µm). The microvessels were treated with $\alpha_v\beta_3$-targeted microbubbles and 2 MHz ultrasound pulses of 10×10 or 10×1000 cycles, evenly spread over 30 s, and peak negative pressures ranging from 55-480 kPa. Controls included non-treated, microbubbles only, or ultrasound only. Permeability changes were investigated using 150 kDa FITC-dextran dye and fluorescent microscopy for 2 h. Cell viability was assessed using a WST-8 colorimetric assay which measures metabolic activity.

Two hours after treatment, vascular permeability was only significantly higher for the microbubble and 480 kPa 10×10 cycles and 350 and 480 kPa 10×1000 cycles ultrasound treatments in comparison to all controls. In addition, within 5 min after treatment only the microbubble and 350 and 480 10×1000 cycles groups showed a clear leakage increase, suggesting an earlier onset of the treatment effect upon the 10×1000 cycles. Furthermore, the plateau of the leakage approached 100% for the 10×1000 cycles with microbubble groups whereas this was ~70% for the 480 kPa 10×10 cycles, indicating that the barrier loss was less with the short cycle's treatment. The spatial leakage was unevenly distributed over the vessel which suggests that some vessel regions were more affected by the treatment than others. Finally, all treatments did not affect cell viability. These results show the potential of a microvessel-on-a-chip to investigate the mechanism and maximize the outcome of ultrasound and microbubble-mediated drug delivery treatments.

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Patient specific cardiac model of dilated cardiomyopathy in a beating heart-on-chip

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Dilated Cardiomyopathy (DCM) is the most common non-ischemic cardiac disease, whose current treatments only slow down its progression without repairing the produced damage (Maron et al., 2006). Elucidating the mechanism of DCM is crucial for the development of efficient therapies. Organs-on-chip (OoC) technology has emerged as a promising tool to replicate *in vitro* the human pathophysiology, allowing the integration of electromechanical stimulus and sensors (Sun et al., 2012). In this study, we generated 3D cardiac models within a heart-on-chip platform (Visone-Lozano et al., 2022) by using induced pluripotent stem cells derived cardiomyocytes (iPSC-CMs) differentiated from a patient presenting

a DCM mutation and from isogenic control. The microtissues were used to investigate the effects of mechanically-active environment on pathological cells and to conduct pharmacological studies.

The PDMS heart-on-chip devices are formed by three layers: an upper actuation layer, which applies a 10% mechanical stretching to the microtissue; an intermediate chamber where cells are cultured and electrodes are embedded; and a bottom coverslide. iPSC-CM were obtained using an established protocol (Lian et al., 2013) and embedded in fibrin at a concentration of 75-120·10^6 cells/mL before injection into the platforms. Microtissues were cultured 7-10 days under static or mechanical conditions and their beating properties were evaluated. Several drugs that block Ca^{2+} channels were tested, and calcium transients and electrophysiological parameters were assessed.

DCM and isogenic iPSC were successfully differentiated and cultured into the organ-on-chip. The beating rate of the DCM microtissues was reduced upon mechanical stimulation (64.71 ± 8.00 to 46.69 ± 19.82 bpm), whereas the biological control was paced at the stimulation rate (from 71.39 ± 24.05 to 57.85 ± 13.19 bpm). Calcium uptake upon stimulation was prolonged in the control cell line (152.23 ± 23.94 in static vs 139.57 ± 8.94 ms in dynamic), while not affected in the DCM microtissues (139.528 ± 17.06 to 139.57 ± 8.94 ms). Administration of 1 nM Nifedipine extended calcium uptake at a higher level in control as compared to DCM conditions after mechanical stimulation (+92% and +25%, respectively).

In conclusion, the DCM model in our heart-on-a-chip can be used to assess the effects of physical stimulation and drug testing on patient-specific cardiac pathologies.

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3D-printed device providing volumetric compression and strain for cortical brain organoids

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Human pluripotent stem cell (hPSC)-derived cortical organoids mimic mature cell types and the cytoarchitecture of the tissue *in vivo*. Still, these organoids lag behind in yielding reproducible, diverse populating cells of the central nervous system (CNS). Volumetric compression and strain have been shown to regulate the intracellular crowding and chromatin structure; respectively [1,2]. Here, we present a 3D-printed device made of Dental SG, a biocompatible material, in a 6-well plate format to be able to apply compression and strain to 3D cortical organoids. The upper part of the device is made of a flexible PDMS membrane (20 µm) that can be actuated to provide on-demand compression and strain to the organoid culture by adjusting the pressure. 3D-printed pillars are also included in the design to ensure medium flow in-between all six wells while the membrane is in complete deflection. The device is also resealable, allowing for easy extraction of the organoids for further analysis off-chip after mechanical stimulation.

References

[1] Jagielska, A. et al. (2017). *Front Cell Neurosci 11*, 93.
[2] Li, Y. et al. (2021). *Cell Stem Cell 28*, 63-78.

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New non-invasive, label-free monitoring approach for 2D and 3D cell culture

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Two major issues of cell-based toxicological and drug response assays are the lack of the temporal component of endpoint assays, and the strong dependency of reproducibility and significance on the quality and condition of the cells used. Thus there is a tremendous need to provide insight into the usually inaccessible processes inside the incubator. We developed a novel lensfree imaging method exploiting the optical properties of the cell itself for imaging inside the incubator, which allows non-invasive, super compact, label-free, live-cell monitoring. By applying AI to determine key cell culture parameters such as confluence, proliferation, and cell motility [1], high-quality, automated, objective, and real-time data can be collected. Applying our lensfree microscopy (LM) method, we find that memory effects from heterogeneous cell culture conditions lead to an increase of variance during subsequent assays like e.g. omics-readouts [2] or other cell based assays, like wound healing assays, motility and proliferation assays significantly. Furthermore, our LM is also suitable for 3D applications and will enable quantification of organoid growth dynamics and interactions. Our approach dramatically increases control and processing speed. In the context of the reproducibility crisis, we hope to make a contribution in the direction of standardization of cell-based research in the future.

References

[1] Rempfler, M. et al. (2018). Tracing cell lineages in videos of lens-free microscopy. *Med Image Anal 48*, 147-161.
[2] Bortel, P., Skos, L., Hagn, G. et al. (in preparation). Multilevel omics-readouts of perturbation studies are determined by memory effects from subculture.

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Using a complex NASH *in vitro* model for drug development with siRNA technology

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Non-alcoholic steatohepatitis (NASH) is a silent and fibrosing disease of the liver which lacks curative therapy and is the leading cause for liver transplants. Traditional monoculture 2D-models cannot be used to investigate novel potentially therapeutic NASH biology, that is driven by communication across different cell types. Physiologically relevant *in vitro* systems are needed for proof of concept studies of novel NASH treatments, particularly where target biology differs greatly between humans and *in vivo* models. We explored whether the "NASH in a box" model by CN Bio can be used to study siRNA-mediated knock-down of targets for anti-fibrotic NASH therapy.

Human primary hepatocytes, hepatic stellate cells, and Kupffer cells (not donor-matched) were cultured in a microfluidic, circular system for 14 days under fibrosis-stimulating conditions (lipotoxic, glucotoxic, TGFβ) as described in the "NASH in a box" kit. For Knock-down (KD) treatments, siRNA against 2 targets with GalNac-mediated uptake was added to the culture on the seeding day. KD efficiency was evaluated on day 10 and day 14 by qPCR or RNAseq, and fibrotic phenotypes were analysed on mRNA level, immunohistochemistry, and protein read-outs from the supernatant.

SiRNA-mediated KD were efficient (< 25% scrambled mRNA) and stable for 14 days of culture. KD was also stably achieved on the protein level. Viability was stable and unaffected by the GalNac-mediated uptake of siRNA and KD. KD of one target resulted in reduced fibrotic markers, such as pro-collagen secretion, and reduced anti-COL1A1 staining.

We believe that this *in vitro* model can inform drug development on novel targets to treat NASH based on anti-fibrotic mechanisms.

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Development of an innovative cartridge bioreactor for parallelized cultivation and mechanical stimulation of complex tissue models

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Musculoskeletal diseases are among the most common health problems and their incidence continues to rise due to the ageing population. Adequate patient care is only possible if suitable new therapeutic strategies pass through the preclinical and clinical phases and reach the market. Particularly in the preclinical stage, animal models are still being resorted to, as no sufficiently suitable *in vitro* models are yet available. To close this gap, we developed a preclinical 3D *in vitro* model simulating the initial phase of fracture healing by co-cultivating fracture hematoma models (FH) with bone models consisting of self-assembled mesenchymal stromal cells for 48 h. Although under non-perfused and unloaded conditions, our model already demonstrates distinct overlaps between *in vitro* and *ex*/*in vivo* characteristics [1].

Since mechanical loading significantly influences the musculoskeletal system, we developed a multimodal bioreactor capable of applying biomechanical loading allowing us to cultivate 3D models *in vitro* under controlled and constantly monitored conditions.

Therefore, we (i) defined mechanical loading conditions and (ii) applied these conditions to *in vitro* 3D models to resemble the *in vivo* situation. Using this system, (iii) we mimic loading on models of bone regeneration. Finally, (iv) we test biomaterials and medication strategies under defined loading and environmental parameters.

Technically, we developed an insert-based cultivation chamber to incubate four models in parallel. We included a click-on pressure system to implement mechanical loading using a pneumatic valve. Using this system, we could prolong the surviving capacity of the cells in the *in vitro* 3D fracture hematoma models (FH) and accelerate the calcification of bone models consisting of self-assembled mesenchymal stromal cells. Our next step will be to use pellets of mesenchymal stromal cells to reveal the impact of mechanical loading on osteogenic processes.

The multimodal bioreactor proposed here will allow us to (i) cultivate the model for a longer time under perfused and hence closer-to-*in*-*vivo* conditions in order to study the transition of fracture healing from the inflammatory towards the anti-inflammatory

phase and (ii) mimic loading conditions that influence the fracture healing process.

Reference

[1] Pfeiffenberger, M. et al. (2021). *J Bone Miner Res 36*, 1189-1201.

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3D manufactured micro-physiological system for modeling tissue-to-tissue barriers

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Organ-on-a-chip technology recapitulates the key structure and tissue-specific function of human organs with greater reproducibility than existing 3D cell culture models, enabling more accurate modeling of human pathophysiology *in vitro*. However, it remains to be achieved to standardize device manufacturing processes and tissue model construction protocols for modernizing drug development. Here, we show a manufactured microphysiological system to reproduce tissue-to-tissue barriers with key structures and functions in a 3D cellular network.

MEPS-TBC or the tissue-to-tissue barrier chip manufactured by high-precision injection molding has two compartments separated by a porous membrane on which the vascular endothelium resides with shear stress and beneath which 3D perivascular multicellular network is formed. The 3D double layers of MEPS-TBC are designed to be mechanically assembled, and the well-type bottom reservoir allowed easy loading of 3D matrices, including hydrogel-cell mixtures, cell aggregates, organoids, and patient tissue samples. This chip is used to mimic the human blood-brain barrier (BBB) and neurovascular unit (brain), the hepatocyte-endothelial barrier (liver), the dermis-endothelial barrier (skin), the small airway (lung), the glomerular filtration barrier (kidney), and patient-derived tumor microenvironments.

We will highlight our model or MEPS-BBB of the human BBB established on MEPS-TBC, in which an induced pluripotent stem cell-derived brain microvascular endothelial cell (iPSC-BMEC) monolayer is formed in the apical compartment and primary human pericytes and astrocytes in the 3D perivascular region. The 3D BBB model with iPSC-BMEC reconstituted robust barrier tightness, resulting in higher transendothelial electrical resistance (TEER) values and lower permeability coefficients than other *in vitro* BBB models. The flow-induced, specialized BMEC increased the expression of junctional and transporter proteins, and the 3D astrocytic network reduced reactive gliosis and polarized aquaporin-4 distribution. More importantly, the cellular barrier function of our BBB model enabled the characterization of receptor-mediated transcytosis of drugs and nanoparticles and homeostatic trafficking regulation against inflammatory peripheral immune cells.

Our 3D manufactured tissue-to-tissue barrier chip provides a highly reproducible 3D microphysiological system platform for recapitulating key organ-level functions of physiological barriers. Our platform will offer a paradigm-changing, drug-testing platform that can advance alternative tests to existing animal studies for modernizing drug development.

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A 3D lung tumor on a chip model to study the modulation of T cell infiltration under flow conditions in a high-throughput microfluidic culture system

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Immunotherapy is a growing focus field in the battle against cancer, where the immunotherapy focusses on the patients' own immune system to treat tumors. Although there is a correlation between increased T cell infiltration and a positive outcome for the patient, unfortunately a subset of patient tumors demonstrate resistance to existing immunotherapies.

It is widely recognized that data from high-throughput screening (HTS) of anti-cancer compounds on 2D multi-well tissue culture plates does not correlate consistently with clinical results. 3D microfluidic *in vitro* models offer the potential to avoid the labor and cost of *in vivo* rodent models. They overcome species-specific immunological differences between animals and humans which hampers translatability and enhance the complexity over standard *in vitro* systems.

Here we introduce a high throughput (n = 40 chips) 3D lung tumor-on-a-chip model. In the OrganoPlate® 3-lane, an endothelial vessel is formed against a collagen I extracellular matrix. T cells

can be added into the endothelial vessel under flow conditions and infiltrate into a tumor compartment.

First, stability of a tumor/vasculature model using HUVEC and HCC827 tumor cells was optimized in the microfluidic OrganoPlate® 3-lane system. Next, an immune component was added to the culture. Transendothelial migration and infiltration of the T cells was studied in a response to several chemokine gradients, such as CXCL11 and CXCL12. This chemokine gradient was confirmed by medium sampling of the endothelial tube and tumor compartment. A dose-dependent effect on migration was observed for CXCL12. Furthermore, CXCL12 was shown to be more potent for recruiting T cells than CXCL11.

This model can be used to assess the infiltration in a response to chemokine gradients, such as CXCL11 and CXCL12. The 3-lane OrganoPlate® design is composed of 40 chips allowing for the screening of components manipulating this immune response. Furthermore, manipulation of the vasculature by different compounds could help in the search for potential new treatments affecting the infiltration of immune cells into the tumor.

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Organ-on-chip device suitable for anaerobic conditions and decreased drug absorption

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Polydimethylsiloxane (PDMS) is golden-standard material for microfluidic device fabrication, due to its elasticity, transparency, biocompatibility, ease of fabrication, castability and cost [1-3]. PDMS disadvantages involve high gas permeability, which can interfere with providing appropriate anaerobic environment, and small hydrophobic molecule absorption, including several drug molecules, which can lead to obtaining of incorrect data for drug activity and toxicity [2,3].

Herein, we present a novel fabrication method of PDMS-free devices, with a decreased small molecule absorption, and transparency comparable to glass. Off-stoichiometry thiol-ene (OSTE) and cyclic-olefin copolymer (COC) have low oxygen permeability and low small molecule absorption compared to PDMS, which can be beneficial for ensuring hypoxic environment and acquisition of accurate drug activity and toxicity data.

Our OOC devices are fabricated using soft-lithography method from OSTE and COC slides. Our chip consists of top and bottom channels, which are separated by porous PET membrane.

The optical transmission, absorption of 3 drug molecules (Aspirin, Clindamycin, Axitinib) with different logP (hydrophobicity) values and biocompatibility was evaluated for our OOC devices and compared to PDMS devices.

To create an anaerobic gut on a chip (GOC), CACO-2 cells are seeded on the top channel and HUVECs on the bottom channel. After villi formation hypoxic media is used to seed the top channel with $1*10^7$ cfu/ml. CACO-2 cells formed a tight monolayer and after 5 days of culture we could observe villi structures both with bright field imaging and with tight junction fluorescent labelling.

OSTE has a clear advantage over PDMS in small molecule absorption, gas permeability and bonding. COC slides ensure optical transmission comparable to glass and mini Luer ports for easy fluid tubing connection. The functionality of our chips was confirmed with a GOC culture and an anaerobic microbiota culture with over 60% bacterial viability after 24 h.

References

[1] Campbell, S. B., Wu, Q. et al. (2021). *ACS Biomater Sci Eng 7*.
[2] Gokaltun, A., Kang, Y. B. A. et al. (2019). *Sci Rep 9*.
[3] van Meer, B. J., de Vries, H. et al. (2017). *Biochem Biophys Res Commun 482*, 323-328.

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Contractile force measurement in a beating heart-on-a-chip

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Introduction: Organs-on-chip aim at mimicking fundamental organ-specific dynamic conditions in microfluidic cell culture devices. In particular, heart-on-a-chip devices are advanced *in vitro* platforms that foster the development and maturation of cardiac micro tissues. Hence these devices potentially represent powerful human models for the prediction of drug toxicity [1].

Building upon a mechanical training platform [2], we here aim at achieving a reliable cardiac model able to assess the contractile force exerted by the cardiac muscle cells upon drug administration (e.g. isoprenaline). Cardiac force is measured by capturing the displacement of two pillars during tissue contraction.

Methods: Device design and fabrication: Layouts of the microfluidic devices were drawn in CAD software and the master molds were fabricated in a cleanroom environment using conventional photolithography techniques.

Human cardiac model generation: Human cardiac models were generated by combining hiPSC-CMs and adult human dermal fibroblasts in a ratio of 3:1, respectively. Cells embedded in fibrin gel (10 mg ml^{-1} of human fibrinogen and 1.25 U ml^{-1} of human thrombin) at a density of 125×10^6 cells ml^{-1} were seeded in the devices and cultured for 7 days (5 of which under dynamic conditions).

Contractile force measurement: Muscle contraction force was estimated by measuring the deflection of two pillars during contraction. Recorded videos were analysed using a custom Matlab script, and the contraction force was obtained using Eq. 1 [3]: $F= 3EI\delta/L^3$ (1), where E is the young's modulus of PDMS, I the second moment of area, δ the pillar displacement, and L the pillar height.

References

[1] Visone, R. et al. (2021). Micro-electrode channel guide (μECG) technology: an online method for continuous electrical recording in a human beating heart-on-chip. *Biofabrication 13*, 035026. doi:10.1088/1758-5090/abe4c4

[2] Marsano, A. et al. (2016). Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. *Lab Chip 16*, 599-610. doi:10.1039/C5LC01356A

[3] Uzel, S. G. M. et al. (2016). Microfluidic device for the formation of optically excitable, three-dimensional, compartmentalized motor units. *Sci Adv 2*. doi:10.1126/sciadv.1501429

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Bridging the gap – how human microphysiological systems improve the translatability of NASH drug discovery

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Non-alcoholic fatty liver disease (NAFLD) is one of the most prominent forms of chronic liver disease worldwide, reflecting the epidemic of global obesity. Those with progressive variant of NAFLD, non-alcoholic steatohepatitis (NASH), are at significantly greater risk of multisystem morbidity and mortality. Traditionally, preclinical trials are mainly based on 2D and *in vivo* animal models but have failed constantly to predict human drug efficacy as they lack translatability and inadequately recreate the complexity and multifaceted nature of this human disease. Building more predictive, human-relevant models is crucial to successfully bringing efficient anti-NASH therapies to the market.

Microphysiological systems (MPS) recapitulate key aspects of human organs' phenotype and architecture. MPS models are currently undergoing rigorous validation studies so that in the future they may be fully adopted as standard preclinical assessment tools. The PhysioMimix™ NASH model captures all key aspects of the human disease: intracellular hepatic fat accumulation, inflammation (secreted cytokines and chemokines such as IL-6, IL-8 and TNFα) and fibrosis (extracellular matrix components and profibrotic markers such fibronectin, and TIMP-1). Recently we validated our advanced MPS NASH model using two anti-NASH compounds, obeticholic acid and elafibranor. Both compounds matched clinical findings by significantly reducing inflammatory and fibrosis markers.

Here, we further expand the validation of our NASH model by measuring the effects of two more anti-NASH compounds that are currently in late-stage NASH/NAFLD clinical trials. Selonsertib showed no antifibrotic or anti-inflammatory effects in our MPS NASH model at clinically relevant dosage, matching results from phase III STELLAR trials, despite reduced fibrosis and inflammation being detected in alternative 3D spheroid models. Aramchol showed a significant reduction in fibrosis (matching data from ARMOR Phase III study) and proved to be safe at highest tested concentration without altering liver microtissues' functionality. Overall, we demonstrate how this NASH liver MPS provides translatable insights into drug efficacy for NASH therapeutics and brings a promising, sensitive alternative for pre-clinical NASH screening to help fast-track decision making and access to the market.

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Investigating the contribution of cartilage and synovium to osteoarthritis development through a compartmentalized human joint-on-chip model

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Osteoarthritis (OA) is a degenerative joint disease causing pain and disability, for which reversing therapies have not been developed yet, due to the lack of understanding on initial disease mechanisms [1]. In this regard, organs-on-chip can be used to unravel the interactions between joint tissues during OA early stages. To address this, we developed a compartmentalized joint-on-chip allowing for the co-culture of cartilage and synovium tissues, aiming at assessing their contribution to OA pathogenesis.

The proposed platform comprises two culture areas, designed for synovium and cartilage cultures, whose communication is controlled through normally closed valves. Additionally, an actua-

tion layer allows to apply a mechanical compression to cartilage compartment [2]. Human articular chondrocytes embedded in fibrin gel were cultured for two weeks and cartilage maturation was demonstrated by deposition of matrix rich in collagen type-II and aggrecan. A cyclical hyperphysiological compression (HPC) was then applied for one week to induce a shift towards an OA phenotype, as assessed through upregulation of inflammatory markers (e.g. *IL6*, *MMP13*). Human synovial fibroblasts and macrophages embedded in a mix of fibrin gel and collagen type-I were cultured for up to 7 days, and synthesis of collagen type-I and lubricin was shown. Upon valves opening, induction of synovial inflammation due to communication with HPC OA cartilage tissue was demonstrated by the enhanced synthesis of MMPs operated by SFBs and by macrophage polarization towards pro-inflammatory state M1. Moreover, a protocol was defined to induce an inflammatory state in the synovium through TNF-α and IFN-γ, and the role of an inflamed synovium on triggering cartilage degradation is currently under evaluation.

In summary, the compartmentalized microfluidic platform offers a solution to independently mature 3D cartilage and synovial constructs, as well as to induce OA traits in one compartment specifically, through the implementation of temporal control over chambers communication. The device was used to demonstrate that mechanically-induced damages to cartilage triggers inflammatory changes in the synovium. Further studies will help to determine the dominant role of the two tissues in early OA.

References

[1] Bartolotti, I. et al. (2021) *J Clin Med*.
[2] Occhetta, P. et al. (2019). *Nat Biomed Eng*.

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Brain organoids to model human brain diseases

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Understanding how the human brain functions in health and disease is one of the greatest challenges of modern science, yet hindered by limited availability of human samples and ethical restrictions. The three-dimensional human brain organoid model has emerged as a cutting-edge, genetically-tractable experimental system to study human brain development and function in health and disease. Here, I will present one exemplary project illustrating how brain organoids can be employed to model human disease, specifically viral encephalitis.

Herpes simplex encephalitis (HSE) is a life-threatening disease of the central nervous system caused by herpes simplex viruses (HSV). However, with the standard anti-viral treatment, most patients still experience various neurological sequelae. We employed human brain organoids to model acute HSV-1 infection in a complex neuronal tissue and preformed single-cell RNA sequencing, electrophysiology and imaging to characterize the molecular changes associated with HSV-1 infection. We observed strong perturbations of tissue integrity, neuronal function and cellular transcriptomes. Antiviral acyclovir treatment alone, which reflects clinical treatment, prevented viral replication, but did not rescue HSV-1-driven defects observed in organoids. Using organoid model, we further tested alternative treatments for the acute infection to improve current therapeutic strategies.

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3D spheroids of the pancreatic beta cell line EndoC-βH5 for modelling diabetes mellitus in a microphysiological system

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Type 2 diabetes mellitus (T2DM) is a complex multi-organ disease characterized by impaired glucose homeostasis. In healthy individuals, pancreatic β-cells respond to increased blood glucose concentration by secreting insulin. To date, multi-organ diseases, such as T2DM are not adequately reflected in animal models or in standard *in vitro* models. To address this challenge, we have recently shown functional coupling of human HepaRG spheroids and human islet microtissues [1,2] for studying the interplay between the pancreas and the liver, two key organs in blood glucose regulation. Here we show spheroids made of EndoC-βH5 cells as an alternative pancreas organ model.

The human pancreatic β-cell line EndoC-βH5 exhibits a near-native β-cell phenotype. The cells are available as ready-to-use frozen stocks, which have high batch-to-batch reproducibility and maintain their function for weeks [3]. We first optimized a protocol for spheroid formation and observed stable morphology and stable glucose-stimulated insulin secretion (GSIS). The

EndoC-βH5 spheroids showed dose-dependent insulin response to glucose and, moreover, stimulation with the GLP-1 receptor analog exenatide led to a significantly increased insulin secretion. We then demonstrated that the EndoC- βH5 spheroid model can be co-cultivated together with HepaRG spheroids in the HUMIMIC Chip2 for up to 15 days. Liver function, as shown by albumin secretion, was stable over time. EndoC- βH5 cell functionality was demonstrated by insulin release into the culture medium as well as by GSIS of spheroids extracted from the chips at the end of the culture period. Organ cross-talk was investigated by an *in vitro* glucose tolerance test measuring glucose, lactate and insulin concentrations in response to a glucose load.

The newly developed 3D spheroid model of EndoC- βH5 cells represents a reproducible and functional human β-cell model to study the liver-pancreas axis *in vitro*. In the future, the established co-culture model could be used as a platform for studying human islet biology in both healthy and T2DM as well as for development of new therapies.

References

[1] Bauer, S. et al. (2017). *Sci Rep 7*, 14620.
[2] Casas, B. et al. (2022). *PLoS Comput Biol 18*, e1010587.
[3] Olleik, H. and Blanchi, B. (2022). *Diabetes 71*, *Suppl 1*, 254-LB.

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Xeno-free bioengineered human skeletal muscle tissues

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Bioengineered human skeletal muscle tissues have emerged in the last years as new *in vitro* systems for disease modelling. These bioartificial muscles are usually fabricated by encapsulating human myogenic precursor cells in a hydrogel scaffold that resembles the extracellular matrix (ECM). However, most of these hydrogels are derived from animal sources, which can limit clinical applications of engineered tissues in regenerative medicine due to the possible presence of xenogenic contaminants from animal-derived ECM. Moreover, animal-derived serum in culture media could reduce sensitivity to drug toxicity within *in vitro* testing platforms. These limitations can be overcome by using xeno-free biomaterials and culture conditions, which offer increased relevance for developing human disease models.

In this work, for the first time, human platelet lysate-based nanocomposite hydrogels (HUgel) were used as scaffolds for human skeletal muscle tissue engineering in a xeno-free culture. These hydrogels consist of human platelet lysate reinforced with cellulose nanocrystals that allow tunable mechanical, structural, and biochemical properties for the 3D culture of stem cells. Here, we fabricated hydrogel casting platforms for the encapsulation of human muscle satellite stem cells in HUgel around a pair of flexible posts, which act as tendon-like anchoring sites that aid cell alignment and myotube formation. The content of cellulose nanocrystals was modulated to obtain long-lasting scaffolds with high clot retraction properties that promote skeletal muscle tissue formation. We demonstrated that this optimization enhanced matrix remodelling, uniaxial tension, and self-organization of the cells, resulting in the formation of highly aligned, long myotubes expressing sarcomeric proteins. The xeno-free bioengineered human muscles were subjected to a frequency sweep electrical pulse stimulation regime. The electrically stimulated tissues presented twitch or tetanic contractions depending on the applied frequencies. Furthermore, the exerted contractile forces were measured in a non-invasive manner.

Overall, our results demonstrated that bioengineered human skeletal muscles could be built in xeno-free cell culture platforms to assess tissue functionality. These tissues could be used as patient-specific models for muscular diseases by incorporating patient-derived platelet lysate and satellite cells. Remarkably, the xeno-free characteristics of this *in vitro* 3D model could also enable the transition into clinical applications for regenerative medicine.

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The effect of embedded macrophages on intravascular coagulation in 3D vessels-on-chips

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Macrophages are innate immune cells that prevent infections and help in wound healing and vascular inflammation [1]. While these cells are natural helper cells, they also contribute to chronic diseases, e.g., by infiltrating the endothelial layer in early atherosclerosis and by promoting vascular inflammation. There is a cross-talk between inflammatory pathways and key players in thrombosis, such as platelets and endothelial cells – a phenomenon known

as "thromboinflammation". The role of the embedded macrophages on thromboinflammation in vascular disease is incompletely understood.

Blood perfusion assays are a good way to determine the state of an endothelial layer in a blood vessel-on-chip model. Multiple research projects have shown the advantages of using blood perfusion assays in blood vessel-on-chip models [2]. These models are usually based on square microchannels that do not represent the *in vivo* geometries of a real blood vessel.

Using induced pluripotent stem cell derived endothelial cells and polarized THP-1 monocytes, we have developed a 3D blood vessel-on-chip with embedded macrophages, which is created using sequential cell seeding in viscous finger-patterned collagen hydrogels. We have set up a human whole blood perfusion assay for these 3D blood vessel-chips.

Platelet formation was seen in brightfield images, but were not observed in fixated samples, due to washing steps to clear the channels of residual blood. An increased formation of fibrin in the blood vessel-on-chip models containing (lipid loaden) macrophages was observed indicating an increasing in the blood clotting process in these channels.

In conclusion, we have developed a 3D vessel-on-chip model with embedded macrophages and have demonstrated that it can be perfused with human whole blood. With the first version of the 3D model established, we expect to use it in future studies of the role of macrophages in thromboinflammation.

References

[1] Shirai, T., Hilhorst, M., Harrison, D. G. et al. (2015). Macrophages in vascular inflammation – From atherosclerosis to vasculitis. *Autoimmunity 48*, 139-151.

[2] Albers, H. J., Passier, R., van den Berg, A. et al. (2019). Automated analysis of platelet aggregation on cultured endothelium in a microfluidic chip perfused with human whole blood. *Micromachines 10*.

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An *in-vitro* platform to test mediators of neuronal death in Parkinson's disease and neurodegenerative disorders

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The death of dopamine-producing neurons in the substantia nigra in the base of the brain is a defining feature in the development of Parkinson's Disease (PD). PD is, however, a multi-systemic disease, also affecting the peripheral nervous system and gastrointestinal tract. Neuroinflammation and neurodegeneration may be exacerbated, or even initiated, by changes in gut microbiota composition and function [1], propagated via intestinal mucosal immune and neural networks [2].

We have developed a microfluidic organ-on-chip system [3] to model gut-to-brain cell translocation of soluble mediators of neuronal inflammation and cytotoxicity. Using a known neurotoxin trigger of PD, 1-methyl-4-phenylpyridinium (MPP^+), we demonstrate that our system effectively reproduces neurotoxin-induced dopaminergic death *in vitro*. Using quantitative and morphometric image analysis methods, we have quantified the resulting impacts on neuronal mitochondrial and nuclear phenotypes, thus demonstrating high-throughput analysis capability and utility of this system for measuring neuronal responses to toxic stimuli

We are now further developing the system to test how various microbially-produced molecules exacerbate, or protect against, neuronal inflammation and death. These molecules may act either directly on blood-brain-barrier cells and dopaminergic neurons, or indirectly via immune and neural gut-brain signalling mechanisms. Thus, we intend that this system will be more widely applicable to the study of pathological mechanisms in the gut-brain axis, and for testing the efficacy and safety of drug candidates of neurodegenerative disorders.

References

[1] Romano, S., Savva, G. M., Bedarf, J. R. et al. (2021). Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *NPJ Parkinsons Dis 7*, 27.

[2] Pellegrini, C., Antonioli, L., Colucci, R. et al. (2018). Interplay among gut microbiota, intestinal mucosal barrier and enteric neuro-immune system: A common path to neurodegenerative diseases? *Acta Neuropathol 136*, 345-361.

[3] Baldwin, L., Iles, A., Greenman, J. et al. (2019). Dual-flow microfluidic device for modelling biological barrier systems. Micro-TAS, Basel, Switzerland.

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Use of organ-on-a-chip technology in preventive doping research to predict possible metabolites for the calstabin-ryanodine receptor complex stabilizer S107

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In preventive doping research, the prediction of potential metabolites of substances banned in sport is essential. This applies to non-approved substances that are assigned to the category S0 of the World Anti-Doping Agency (WADA) Prohibited List, such as the calstabin-ryanodine receptor complex stabilizer S107. In general, various *in vivo* and *in vitro* approaches can be applied to predict metabolite formation and secretion of these substances into urine. Human excretion studies provide the most accurate prediction of metabolites; however, these *in vivo* studies are often difficult to perform with non-approved substances. Another *in vivo* option is to perform animal studies, where transferability from animal model systems to humans is usually limited. 2D *in vitro* cell culture models with human cell lines or experiments with human liver microsomes and S9 liver fractions can provide first indications of metabolites, but are not complex enough to mimic human physiological conditions. Therefore, in the present study, a 3D-liver model and organ-on-a-chip technology was applied for metabolite prediction of the drug candidate S107 by using HepaRG liver spheroids cultured in a microfluidic system. The system was characterized at first for expression and activity of key CYP enzymes. In a second step, the metabolic transformation of S107 was analyzed during a three-week experimental period with repetitive substance application into the system. Supernatants at the experimental time points d3 to d21 were analyzed by HPLC-HRMS/MS to identify the metabolites formed. The metabolic profile was compared to metabolites detected in previous *in vitro* studies to investigate the applicability of the organ-on-a-chip system for anti-doping research.

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Tooth on a chip: An MPS to mimic innervated dental pulp and mineralised interface

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Dental pathologies are widespread within the population and can lead to significant functional issues and tooth loss. Traditional treatments, which involve replacing damaged tissues with artificial substitutes can result in reduced tooth strength and post-operative complications. Regenerative strategies, which adopt stem cells and environmental or pharmacological stimuli to restore functional tissue, may offer a solution to these limitations [1].

A particular stem niche resides in the dental pulp which is a highly vascularized and innervated connective tissue characterized by dental pulp stem cells (DPSCs) and an outermost layer of odontoblasts in direct contact with dentin. Odontoblasts maintain dentin homeostasis and protect the pulp tissue, whilst DPSCs are a multipotent mesenchymal stem cells (MSCs) population exerting a pivotal role in tissue development and repair/regeneration processes [2]. DPSCs exhibit multipotency and immunomodulation capacity, but even though encouraging results are being achieved, their clinical application still faces many challenges. These refer to a lack of a profound understanding of their microenvironment, biology and functionality *in-vivo*.

Organ-on-chip (OoC) technology has been recently proposed as a powerful tool to replicate the complex *in vivo*-like microenvironment, opening new opportunities for MSC translation in clinical therapy and for advanced drug testing platforms development. With these purposes, the aim of this study was to create a 3D miniaturised dental pulp *in vitro* model by co-culturing multiple cell lines to reproduce dental pulp organization and physiology. In particular, the OoC hosted dentin and a variety of cells reproducing pulp components: primary human DPSCs, human umbilical endothelial cells (HUVEC), Odontoblast-like cells (Ob) and embryonal mouse trigeminal ganglia (TGG). Co-culture conditions were optimised and resulted in high viability, proliferation, and maintenance of DPSCs stemness. HUVEC self-assembled in a vascular-like structure with DPSCs acting as pericytes and TGG axons were able to innervate the vascularised pulp tissue. Moreover, Ob proved to preserve their phenotype and arranged along a polarised direction. In conclusion, the designed device is a valuable tool for dental microanatomy modelling and can be potentially used in the landscape of a preclinical

dental tissue research to study dental tissue development, pathological pathologies and regenerative processes.

References

[1] doi:10.22203/ecm.v029a16
[2] doi:10.1038/s41598-018-37981-x

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An open-top OoC-platform to generate a fully hiPSC-derived model of the outer blood-retinal barrier with a functional microvascular network

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Pathological changes within the outer blood-retinal barrier (oBRB) are associated with retinal diseases such as age-related macular degeneration (AMD). Degradation of the vascular layer (choroid) and atrophy of the retinal pigmented epithelium (RPE) are the most severe changes correlated to AMD [1]. To better understand the underlying mechanism of AMD, physiologically relevant three-dimensional *in-vitro* oBRB models are needed. Here, we present a fully hiPSC-derived oBRB on-chip model that mimics the physiological crosstalk among the cells of the human oBRB.

A microphysiological platform was designed to enable fabrication by employing off-the-shelf products without using expensive "cleanroom" facilities. The device was made of a micromachined slab of polymethylmethacrylate (PMMA), containing nine individually addressable microfluidic chips. Each chip consisted of an open-top hydrogel chamber flanked by two medium channels. Each medium channel was connected to the gel chamber by pillars and two medium reservoirs. A cell-laden hydrogel was loaded in the gel chamber by pipetting through the open top in the center of the chamber. Nutrients were supplied by interstitial flow generated by a pressure gradient established between the channel medium reservoirs.

By embedding hiPSC endothelial and vascular smooth muscle cells [2] in a fibrin-collagen hydrogel, a self-assembled and functional vascular bed could be generated under flow conditions within 2 days, indicated by the perfusion with fluorescently labeled 10 µm beads. The open-top design of the OoC-platform enabled the direct seeding of hiPSC-derived RPE [3] cells on top of the vascular bed, which developed a confluent monolayer within two days of co-culture. After a total of 8 days, the RPE apically expressed Ezrin 1, a marker associated with the maturation of RPE cells. Additionally, the tight junction marker ZO-1 was expressed indicating a tight epithelial barrier.

We expect that the described model will enable the investigation of mechanisms related to retinal diseases *in-vitro*.

References

[1] Brinks, J. et al. (2022). *Prog Retin Eye Res 87*, 100994.
[2] Vila Cuenca, M. et al. (2021). *Stem Cell Reports 16*, 2159-2168.
[3] Regent, F. et al. (2019). *Sci Rep 9*, 10646

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3D-printed human hair follicle model to investigate topically administered nano-antibiotics

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Hair follicle infections with biofilm forming bacteria are difficult to treat [1], demanding novel therapeutic approaches. Drug-loaded nanocarriers are able to penetrate hair follicles [2,3], providing novel approaches for targeted topical delivery to skin appendages, e.g., in the case of diseases like Acne Inversa or Folliculitis Decalvans. However, measuring biological effects of follicle-targeted nanocarriers in *ex vivo* models such as pig ears is difficult, because of limited vitality. Existing *in vitro* models use "floating" biopsies of single hairs and are thus not suitable for studying the penetration of nanocarriers. The objective of this work is to design a 3D *in vitro* organ culture of human hair follicles, as a platform to apply topical transport systems against hair follicle infections. Human anagen hair follicles were isolated from skin biopsies originating from cosmetic

surgery. Then, they were implanted perpendicularly into a collagen matrix within a 3D printed PCL scaffold with nutrition channels, emulating the dermis. Hair growth was measured over 7 days and compared to conventional cultured hair follicles, free floating in medium. High similarity of hair growth was shown for both approaches. To mimic infected hair follicles, which can be treated, the model was infected with *Staphylococcus aureus*. Fluorescence microscopy showed colonization of the hair follicle post infection and inflammation mediators were detected in the surrounding medium, which was not the case for non-infected, healthy controls. As a proof of concept, the application of fluorospheres (200-1000 nm), added on top of the model, were tested for follicular transport. Confocal images indicated size dependent follicular transport of nanoparticles.

This model could serve as a platform to test topical transport systems, like nano-antibiotics to face bacterial hair follicle/skin infections. A further development of the system towards higher complexity could add an epidermis substitute or human cells, like fibroblasts, keratinocytes or immune cells.

References

[1] Polak-Witka, K. et al. (2019). *Exp Dermatol 29*, 286-294.
[2] Christmann, R. et al. (2020). *J Invest Dermatol 140*, 243-246.e5.
[3] Lademann, J. et al. (2007). *Eur J Pharm. Biopharm 66*, 159-164.

Presentation: Oral

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Realization of an organ-on-chip for the study of gastrointestinal chronic diseases, carcinogenesis and tumors

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Intestine-on-chip models able to mimic the organ pathophysiological mechanisms and microenvironment have the potential to replace animal experimentation and speed up research and the pharmaceutical development phase [1-3].

Here we report on the development of an Intestine-on-chip model to investigate and support the treatment of both chronic and cancerous gastrointestinal diseases. The device was constructed through photolithography and soft lithography techniques, taking advantage of the biocompatibility and transparency of polydimethylsiloxane (PDMS). The miniature platform consists of two overlapping PDMS layers separated by a porous membrane to recreate the epithelium-endothelium interface, each containing a channel with an inlet and outlet and two vacuum chambers to mimic the peristaltic movements of the intestine. Both PET and polycarbonate membranes were used, and it was seen that device closure is better when PET membranes are employed since the plasma oxygen treatment used to seal the device is more effective on PET than on polycarbonate. In addition, to optimize device closure, a mortar layer was used on the PDMS replica consisting of a mixture of PDMS and toluene (5:3). Then the microfluidic platform undergoes heat treatment at 90° for 4 hours, having small 50-g weights on top of the chip to enhance clamping. As for the cellular part, a human colorectal adenocarcinoma cell line (Caco-2) was used, which is the most widely used in the literature. These are seeded on the membrane (75 x 10^3 cells/ml), coated with extracellular matrix proteins (e.g., collagen I), through a syringe pump, so that the chip is always perfused and both adequate nutrient supply and removal of metabolic waste products are always ensured. The tightness of the barrier was investigated and potential drugs were screened.

References

[1] Kim, H. J. et al. (2012). *Lab Chip 12*, 2165-2174. doi:10.1039/C2LC40074J
[2] Shin, Y. C. et al. (2020). *Micromachines 11*, 663.
[3] Monteduro, A. G., Rizzato, S., Caragnano, G. et al. (submitted). Organs-on-chips technologies – A guide from disease models to opportunities for drug development.

Presentation: Poster

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Bone-on-a-chip: Culture of human 3D bone-like organoids in a self-designed microphysiological system to model intramembranous ossification

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Organoid culture in microphysiological systems (MPSs) is a promising alternative not only to monolayer cell cultures of limited physiological relevance, but also to the still common animal experiments, whose species-specific differences lead to difficulties in transferability of data to human physiology [1]. Compared to

MPSs found in literature for modeling lung, liver or kidney, bone models are still underrepresented and rather simplistic.

With the aim to model the different steps of intramembranous ossification (IMO) in a more physiologically relevant way than in previously existing models [2], we have generated several human three-dimensional (3D) *in vitro* models and we have cultured them in a MPS. To this end, we have included scaffold-free and scaffold-based bone-like organoids originating from primary osteoblasts (OBs) in a self-designed MPS that, in addition to permanent perfusion, can stimulate organoids with mechanical loading, and reduce oxygen tension (rO2) to a physiological range for bone (7% to 12%) [3].

We have developed several protocols for the generation of bone-like organoids based on i) self-assembly to mimic the starting point of IMO, ii) collagen scaffolds to investigate the onset of bone matrix mineralization, and iii) bio-printed hydrogels to study the conversion of OBs to osteocytes (OCY). All models were maintained in the MPS for seven days with either i) perfusion at atmospheric oxygen concentration, ii) perfusion with additional mechanical loading, iii) perfusion at rO2 or iv) perfusion at rO2 with additional mechanical loading. The established culture conditions allowed survival of the three kinds of bone-like organoids and the evaluation of osteogenic parameters such as alkaline phosphatase, procollagen, prostaglandinE2, nitrite as a downstream product of nitric oxide, bone matrix mineralization, and osteocyte-specific prolonged cell processes besides measurements of general metabolic parameters.

We expect that future applications of 3D bone-like organoids cultured in MPSs will have the potential to replace animal testing in basic and applied research in the context of bone biology.

References

[1] Knight, A. (2007). *Altern Lab Anim 35*, 641-659. doi:10.2174/157488708784223844

[2] Scheinpflug, J. et al. (2018). *Genes 9*, 247. doi:10.3390/genes9050247

[3] Keeley, T. P. and Mann, G. E. (2019). *Physiol Rev 99*,161-234. doi:10.1152/physrev.00041.2017

Presentation: Poster

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Preclinical drug delivery testing and evaluation using lung-on-a-chip technology

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Introduction: New drug development is high-risk, time-consuming, and extremely expensive, – even in the initial phases of development. Additionally, preclinical animal studies require skilled professionals and technological expertise, although there is an increased focus on alternatives that could replace animal testing and not only these experiments are expensive, time-consuming, and ethically questionable, but data from these experiments also repeatedly fail to represent response in human. SARS-CoV-2 pandemic drew attention to the necessity to test highly infectious diseases in a more physiologically relevant and speedy setting. Human organ-on-a-chip (OOC) technology offers a potential solution to these problems since they recapitulate the environment of a specific human organ. OOC devices are based on the concept that it is possible to replicate certain functions of a human organ by culturing the relevant human organ cells (e.g., lung epithelial and endothelial cells for simulating lung tissue) in horizontal microfluidic channels separated by a porous membrane and would not require specialized training.

Materials and methods: Drug delivery, particularly cisplatin (CisPt) at concentration 20ug/ml encapsulated in extracellular vesicles (EVs) were tested on a normal lung-on-a-chip (LoC), developed by CellboxLabs. CisPt concentration in EV sample was determined using HPLC. Chips were cultivated in air-liquid interface for 2 weeks before introduction to the drug. CisPt was added to the endothelial cell media and continuously flushed through endothelial channel for 48 h at a flow rate 2 µL/min. Cytotoxicity, tissue integrity, permeability, cell migration and mucus production were compared between LoC with and without EVs+CisPt, Evs alone and CisPt alone.

Results: Preliminary results show that LoC system is a promising preclinical model for drug delivery testing and toxicology research. Preliminary results suggest of EV ability protect lung tissue integrity based on lucifer yellow experiments in comparison to cisplatin alone. The final results will be presented at the conference.

Research was financed from project nr. ERAF 1.1.1.1/21/A/033

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EIS chip for precision medicine: Measuring sorafenib effectiveness on HCC cell proliferation

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The gold standard in the evaluation of the potential anti-cancer drug therapies are cell proliferation assays made by standardized techniques (e.g., MTT assay). However, these methods are time and cost consuming and not able to satisfy the request to design patient's specific therapies *(precision medicine)*.

Here, we report the development of a miniaturized *electric cell-substrate impedance spectroscopy (ECIS)* platform to perform cell proliferation and drug screening assays based on *impedance spectroscopy (IS)* and its application to investigate the time-dependent effect of Sorafenib on hepatocellular carcinoma cells (HLF) [1].

The *Lab on Chip (LoC)* device consists of a sensing module of four arrays with interdigitated microelectrodes as transducers, combined with a printed biocompatible chamber sealed on the chip.

After cells seeding and attachment, the assays were performed in *real time* (about 72 h) by *optical imaging* and *IS measurements* recording the signals at 40 kHz under 1 mV voltage amplitude allowing a good signal-to-noise ratio without affecting the cell growth.

We carried out drug tests starting from results of traditional MTT assay investigating the drug effect at different concentrations (0, 2.5, 5 and 10 μM in DMSO) on cell growth and then, the same experiments were run on *LoC*. Comparing the results, the set of curves exhibited a remarkable dose-dependent behaviour of HLF proliferation.

We performed *multifrequency EIS tests* (1 Hz-1 MHz) by *LoC* to analyse a spectrum of signals derived from cells and, extracting vectors, the results demonstrated the ability to monitor cell growth, vitality, and motion at immobilization sites in real-time. In conclusion, compared to the traditional one, our platform presented several advantages, among these: lesser reagents consumption, operator time, and costs. It could enable high-throughput studies on large drug candidate libraries or on combinations of multiple compounds to assess their putative synergistic effect on patient-derived samples. A further step will be the use of our platform to support *Organ on Chip (OoC)* by TEER experiments and biomarkers detection supporting research, allowing drug screening and precision medicine.

Reference

[1] Piccinno, E. et al. (2021). Validation of a lab-on-chip assay for measuring sorafenib effectiveness on HCC cell proliferation. *Int J Mol Sci 22*, 13090.

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Generation of miniaturized, vascularized microtissues with human extracellular matrix and tumor microenvironment for chemosensitivity testing of head and neck cancers

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Introduction: Head and neck cancer squamous cell carcinoma (HNSCC) is the 6th most common cancer type worldwide and associated with a high rate of mortality (> 50% within 5 years). Because of the lack of predictability of drug effects from current cell- and tissue-based model systems, there is an urgent need to develop and validate physiologically more relevant *in vitro* model systems. Therefore, we describe the development of new, vascularized microtissues that are miniaturized and standardized; rely on entirely human ECM and can incorporate relevant components of the TME such as normal or cancer-associated fibroblasts (CAFs), and later also immune cells.

Hypotheses and experimental procedure: The formation of vascularized microtissues is based on the co-culture of human adipose-derived stem cells (hASCs) and human endothelial cells (HUVECs), which spontaneously deposit an entirely human ECM [2]. This ECM can be decellularized, and re-populated by other human cell types, such as primary cancer cells and CAFs.

Results and discussion: We co-cultured either hASCs or CAFs with HUVECs and added cancer cells. Immunofluorescence (IF) stainings have been performed and show that the HUVECs form tubes, or vasculature with a hollow lumen typical of vascular structures; while tumor cells form various structures, depending on their aggressive nature. Some tumor aggregates form alignments along or in parallel to the vascular tubes, while others form

clusters. Additional studies are in progress to investigate the interaction between tumor cells and vascular capillaries, with a strong focus on monitoring dynamic processes such as tumor cells intravasation and vascular mimicry; and the role of the NOTCH pathway in these processes.

Conclusions: Our results show that we were able to reconstruct physiologically relevant and vascularized tumor microtissues. These hold great potential for advanced disease modeling, such as chemosensitivity drug testing and personalized medicine.

This project was financially supported by NCN OPUS program (no. 2020/37/B/NZ4/03920).

References

[1] Johnson, D. E., Burtness, B., Leemans, C. R. et al. (2020). Head and neck squamous cell carcinoma. *Nat Rev Dis Primers 6*, 92.

[2] Huttala, Outi et al. (2022). Decellularized in vitro capillaries for studies of metastatic tendency and selection of treatment. *Biomedicines 10*, 271.

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Cancer-on-chip assay for paclitaxel sensitivity of breast cancer tissue

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Introduction: Breast Cancer (BrC) response to chemotherapy is variable and biomarkers are not sufficient to correctly anticipate therapy response. Therefore, we aimed to develop an *ex vivo* assay to predict chemotherapy response in BrC patients, using a novel microfluidic platform.

Materials and methods: Patient-Derived Xenograft (PDX) tumors with known *in vivo* paclitaxel sensitivity were sliced and cultured in 6-wells plates (referred to as *ex vivo* culture), or in the Cancer-on-Chip (CoC) platform (BI/OND). Tissue slices were treated with paclitaxel (1-100 nM) under both culture conditions. EdU (3 µg/ml) was added to tissue slices 2 hours prior to fixation to assess cell proliferation. Tissue slices were Formalin-Fixed Paraffin-Embedded (FFPE) and 4 µM tissue sections were immunostained for proliferation, mitosis and apoptosis. Alternatively, a whole-mount immunostaining was performed to compare the 3D architecture of a fixed tissue slice with and without treatment. To observe cells over time a time-lapse experiment was done using Hoechst staining.

Results and discussion: Our assay allows assessment of the sensitivity of the PDX tumors to paclitaxel treatment by determining the ratio between mitotic and S-phase cells. Preliminary data suggest that treatment with 10 and 25 nM paclitaxel allows to distinguish between *in vivo* tested sensitive and resistant tumors. After treatment with paclitaxel a better dose-response curve was achieved under the CoC culture than after the *ex vivo* culture. The optimized 3D imaging methods allowed observation of aberrant behavior, including failed mitosis, after paclitaxel treatment. Preliminary data on the mechanism of paclitaxel sensitivity obtained using these techniques will be presented.

Conclusions and future perspectives: In this study a paclitaxel-sensitivity assay was optimized for breast cancer PDX material. The first data on the predictive power of this assay will be presented. Eventually, a clinical study will be set-up to validate the newly-developed paclitaxel prediction assay. First step was set towards the life imaging of the tissue slice after treatment. More research will be focused towards further optimization of these methods on a CoC platform.

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HLA diversity and compatibility in immunocompetent human tissue models

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The rising development of immunocompetent human tissue models has been met with the challenge of sourcing appropriately Human Leukocyte Antigen (HLA)-matched immune cells. Compatibility of different donor-derived cell sources within such models is often overlooked, leading to potential alloreactivity that can obscure interpretation of the true immune response. Moreover, despite the importance of representing immune diversity and specificity in models of drug efficacy and safety, this is rarely accounted for in preclinical *in vitro* testing. As a result, reports on inter-individual variabilities amongst drug responders and non-responders, and population-specific drug-induced immune-related adverse events (irAEs) are only generated when the drug has entered clinical trials and reached the market.

Here, we provide an overview of the vast haplotype diversity and limited population overlap of the human HLA system based on publicly available allele frequency databases [1]. We give a perspective on how this information, together with routine adoption of next generation sequencing (NGS)-based HLA typing, can guide experimental work towards building more predictive human tissue models. This includes a program-wide initiative to sequence sample

HLA genes, create a repository of results, and pro-actively guide the choice of cell sources in our multi-cellular tissue models. We also present experimental evidence from a panel of HLA-typed donor-derived human T cells co-cultured with different donor-derived human dermal microvascular endothelial cells (mVECs) in an endothelialized chip screen. Treated and untreated T cells were analyzed by FACS and cytokine secretion to correlate T cell haplotype and degree of mismatch with the endothelium on immune response. Together, this highlights the important need to consider HLA matching and diversity in preclinical test systems, and future work may further help the informed selection of donors/cell lines that best recapitulate authentic and potentially haplotype-specific immune responses.

Reference

[1] Gonzalez-Galarza, F. F. et al. (2019). *Nucleic Acids Res 48*, D783-D788.

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Complex 3D models for head and neck squamous cell carcinoma (HNSCC)

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Introduction: Head and neck squamous cell carcinomas (HNSCC) amount to 600,000 cases per year [1].There is a lack of informative *in vitro* cancer models that represent both tumor microenvironment (TME) and extracellular matrix (ECM) of HNSCC. Here, we investigate the importance of tumor/stroma interactions for tumor growth, aggressiveness, chemosensitivity versus acquired chemoresistance. Specifically, we investigated the role of the NOTCH pathway, the activity of the NOTCH receptors and ligands, and changes in the expression of Notch-regulated genes in chemosensitivity versus resistance.

Theory and experimental procedure: We developed an organotypic 3D cell culture system composed of two layers of extracellular matrix (Matrigel/collagen mixtures), embedding a single layer of co-cultured cell types, such as tumor cells combined with normal or cancer-associated fibroblasts [2]. Live/death assays are performed by ethidium homodimer/ calcein AM staining and confocal imaging, supplemented by WST-8 metabolic assay for endpoint analyses. The invasion of cancer cells is induced and supported by fibroblasts, as monitored by real-time life cell confocal imaging. Changes in the expression levels of Notch-related genes were measured by qRT-PCR and Western blot.

Results and discussion: Our data show that the presence of CAFs significantly modulates chemosensitivity and that both cell viability and drug responses versus acquired chemoresistance depend on the expression and activity of Notch pathway activity and Notch-regulated genes in both CAFs and tumor cells. Chemosensitivity was significantly increased when cisplatin-resistant tumor cells were co-cultured with CAFs and treated with both cisplatin and Notch inhibitors such as crenigacestat. Cell survival and emerging resistance were strongly mediated by overexpression of Notch ligands JAG2 and DLL1.

Conclusion: The 3D sandwich model for cocultures can be used as a simplified, miniaturized, and standardized experimental system to mimic both the ECM and TME of tumors, and investigate chemosensitivity.

References

[1] Picon, H., and Guddati, A. K. (2020). Mechanisms of resistance in head and neck cancer. *Am J Cancer Res 10*, 2742-2751.

[2] Robinson, S., Guyon, L., Nevalainen, J. et al. (2015). Segmentation of image data from complex organotypic 3D models of cancer tissues with markov random fields. *PLoS One 10*, e0143798.

Presentation: Poster

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REV*skin*, a skin-on-chip equivalent with advanced blood-flow mimicry, represents a significant improvement in 3D culture models for wound-healing and skin-ageing studies

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Introduction: Due to the increased regulations on animal testing, the use of human skin substitutes has become more prevalent as a means of testing the safety and efficacy of products intended for use on human skin. In the cosmetic and dermatological industries, various methods to replicate human skin are utilized. Currently, 3D reconstructed skin models are deemed the best *in vitro* options for recreating skin structure and morphology. However, these models have limitations in mimicking the skin's physiological functions for efficacy testing.

Objective: The aim of this study is to replace classic static culture models with full-thickness skin-on-chip models that mimic blood flow, providing more functional and realistic models for skin ageing and wound healing studies.

Materials and method: Juvenile human fibroblasts and keratinocytes were used to grow organotypic skin models in REVIVO BioSystems' microfluidic devices using an animal-serum-free medium and a dermal hydrogel. This allowed the human fibroblasts to synthesize a human-relevant dermal matrix.

Skin-ageing model: In the chip, a proprietary treatment was applied in the medium flowing under the skin model during keratinocyte differentiation.

Wound-healing: The full history of the skin barrier function could be retraced by tracking small molecule secreted by the wounded skin in the artificial blood flow over time.

Results: REVskin grown in microfluidic conditions shows significant improvement compared to static methods, with a thicker epidermis, improved differentiation, and stronger expression of skin markers.

Skin-ageing model: The results show a significant decrease in epidermal thickness (approx. 30%) and a decrease in Ki67 and Lamin B1.

Wound-healing model: The presence of specific cytokines secreted in the imitated blood flow is linked to a lack of skin barrier function. The marker is observed during keratinocyte proliferation and differentiation until the barrier function is restored.

Conclusion: By imitating the function of the blood flow in cell cultures, the conditions are improved, and models that reflect ageing and injury can be created, something difficult to achieve with static cell cultures.

Presentation: Poster

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DigiLoCS – A digital liver-on-chip simulator for predicting human metabolism of drugs

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Organ-on-chips (OoC) are designed to represent human physiology *in vitro* more appropriately than animals and hold great promise to humanize drug discovery and research. Their biggest potential lies in the early prediction of clinical outcomes by identifying hazards and lack of efficacy specific to human biology. Despite their potential, OoCs are not yet routinely used in drug development. Their advantage over animal and other *in vitro* models to infer drug-related (kinetic) properties remains to be established.

In preclinical drug development, a key outcome is setting the first human dose and guide the design of dosage regimens to achieve drug concentrations within the therapeutic window. In this regard, quantifying hepatic clearance (i.e. metabolism) and human pharmacokinetics (or ADME) is critical. To maximize translational and predictive accuracy of *in vitro* systems as a replacement of animal *in vivo* models, an integrated approach combining OoC *in vitro* systems with a computational framework is needed.

To address past shortcomings, we present DigiLoCS, a digital liver-on-chip simulator for the simulation of hepatic metabolism. The developed software platform comprises an advanced mathematical description of the underlying biological processes in liver-on-chips. Published time-concentration profiles from 14 compounds were investigated and model parameters (clearance, permeability) were fitted to describe the data appropriately. In a second step, the estimated *in vitro* clearance values were translated to humans. DigiLoCS outperformed the state-of-the-art approach by more than 70% (prediction ratio DigiLoCS = 1.1 ± 0.4; state-of-the-art = 0.6 ± 0.4), offering a more accurate prediction of human pharmacokinetics. This improved accuracy could help identify the right dose and flag potential hazards or failures earlier in the drug development process. Incorporating more PK data would further improve the performance of DigiLoCS. The software platform can be readily applied and modified for other barrier-organs such as the brain or gut.

Presentation: Oral

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Smart Lid for automated sampling and glucose measurement

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In automated cell culture there is a growing need for sampling and monitoring of vital (functional) parameters such as glucose, lactate, and pH. With standard glucose colorimetric detection kits, manual sample extraction and numerous subsequent dilutions are required to determine glucose concentration which is time consuming, error prone, and increases risk for contamination. As part of the EU-project Moore4Medical, CSEM has developed an integrated microfluidic well-plate lid to periodically sample 6µL of culture medium and measure the glucose level in an incubator environment.

A novel Smart Lid has been engineered to examine single and multi-organ experiments in InSphero's Akura™ flow 384 plate. The Smart Lid maintains sterility and simultaneously enables monitoring of the cell culture medium, without leaving the incubator environment. In conjunction with InSphero's Tilter, the Smart Lid can be operated autonomously.

Briefly, 6 µl samples are taken sequentially at 50 µl/min from the 24 fluidic lane's terminal wells with 5% CV precision. The samples are then individually transferred to the in-line electrochemical glucose sensor, with intermittent washing steps. This procedure can be repeated at least 8 times before exhausting the remaining culture medium. Tests in the range of 0-5.5 mMol glucose (sensor specifications up to 25 mM) have shown good sensitivity without noticeable cross-contamination.

To minimize operator costs, the Smart Lid is divided into reusable and disposable parts. All components in contact with the cells are made of disposable plastic and come pre-EtO-sterilized. The reusable parts (3D printed metal fluidics with 27 memory shape alloy valves and housing) can be wiped and sterilized with ethanol.

Technical validation of the Smart Lid and its individual components has been completed while bio-validation are currently ongoing. A validation testing report on the valves, the sampling, as well as the glucose measurement will be presented together with the single and multi-organ experiments to show the potential of the Smart Lid.

Presentation: Poster

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Engineered microvascular networks using controlled hydrogel structuration on-chip

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Background: In vitro models of human microvasculature and corresponding assays and readouts are useful tools to study disease, and for drug development. Such models potentially offer increased throughput and physiological relevance compared to animal models [1]. Self-assembled microvascular networks can be formed on-chip, but suffer from the lack of controllability over network parameters [2]. Scaffold-guided network formation is therefore a promising approach to engineer pre-defined microvascular networks. Here, we report the rapid fabrication of an Organ-on-a-Chip (OoC) device and its immediate application by performing controlled hydrogel structuration on-chip to engineer human induced pluripotent stem cell (hiPSC)-derived microvasculature. Our approach enables facile engineering of microvascular networks with pre-defined parameters and will lead to models that support relevant readouts such as drug transport and vessel leakage.

Methods and results: A rapid OoC prototyping workflow that we recently reported on was employed here for chip prototyping and fabrication [3]. The approach is based on a commercial digital micromirror device (DMD)-based maskless photolithography setup (Alvéole PRIMO). In this work, we utilized the setup for both OoC fabrication and subsequent on-chip hydrogel structuration. Briefly, by employing gelatin-methacryloyl and a photoinitiator, a scaffold with capillary dimensions (20-30 µm) was engineered after which hiPSC-derived endothelial cells (ECs) were seeded and cultured.

The described approach resulted in cell-seedable and perfusable scaffolds. After seeding, confluency was achieved after one day. Capillary-sized lumen diameters as small as 10 µm were achieved. In addition, arbitrary larger-sized vessels could be engineered and assayed to determine their permeability.

Conclusion: We employed a facile approach for rapid chip fabrication and demonstrated subsequent on-chip engineering of microvascular networks with controlled parameters. The level of control allows engineering of capillary-like vessels that will support *in vitro* modeling of vascular structures that include the blood-brain barrier. When combined with a functional leakage assay, our approach will enable disease modeling and drug transport studies.

References

[1] Ingber, D. E. (2022). *Nat Rev Genet*.
[2] Campisi, M. et al. (2018). *Biomaterials 180*, 117-129.
[3] Kasi, D. G. et al. (2021). *Micromachines 13*, 49.

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IPF-on-chip model based on biological membranes

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and severe lung disease characterized by a progressive scarring of the gas exchange airways. Despite two approved drugs that slow the disease progression, there is no effective therapy. Therefore, there is an urgent need to develop relevant models to investigate key mechanisms underlying the pathogenesis of IPF and to identify potential therapeutic targets. Here, we report the development of an IPF-on-chip model that aims at reproducing the biophysical cellular environment of the early and the late stage of lung fibrosis. The model is based on biological membranes supported by a hexagonal gold mesh that forms an array of alveoli with *in vivo*-like dimensions [1].

The membranes are easily produced by drop casting a solution of collagen-elastin (CE) onto a thin gold mesh. A hydrogel membrane is then formed and can be used as support for cells. The CE solution can be also dried out at room temperature within two days to form a vitrified membrane. The developed biological membranes are flexible and can be deflected in three dimensions when exposed to a negative pressure. Their mechanical properties are largely influenced by the fabrication process as well as by the concentration of collagen, the time of drying and the temperature of gelation [2]. Their stiffness ranges from 1 kPa to 170 kPa allow-

ing the reproduction of the stiffness of healthy and fibrotic tissues.

The biological membranes provide good support for cells growth and proliferation. Human primary fibroblasts and human lung epithelial cells were cultured on the vitrified membrane. It was found that fibroblasts treated with TGF-β secreted more collagen and fibronectin than those without treatment. In addition, barrier integrity decreased over time in epithelial cells treated with this profibrotic cytokine. Further studies on the effect of antifibrotic drugs are being conducted.

In summary, this model reproduces some key features of the lung fibrosis alveolar environment in terms of structure, extracellular matrix composition and mechanical properties. Its entire biological nature makes it a promising tool for drug discovery.

References

[1] Zamprogno et al. (2021). *Commun Biol*.
[2] Zamprogno, Thoma et al. (2021). *ACS Biomater Sci Eng*.

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Permeation of small molecules through biological and synthetic skin simulants using an organ-on-chip platform

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The assessment of how active ingredients penetrate the skin is crucial for the development of dermatological products. We have created a novel skin-on-a-chip testing platform that offers efficient screening.

Objectives: This study aims to accomplish two objectives: (1) compare the barrier function of 3D skin equivalents, synthetic membranes, and native skin biopsies, and (2) determine if our skin-on-a-chip system can sustain native skin samples during prolonged dynamic culture.

Methods: Two common dermatological ingredients, caffeine and salicylic acid, were applied to three commercially available skin simulants, two *ex vivo* skin models (porcine and human), and three synthetic membranes using our microfluidic permeation devices (REVex chips). The automated permeation tests were conducted on the ReleGO system using for 20 hours with infinite and finite doses. The viability of human skin samples cultured dynamically was also assessed over 15 days.

Results: For caffeine, the commercial skin simulants showed permeability coefficients that were one to two orders of magnitude higher than human skin explants, while porcine skin and silicone membranes mimicked human skin's barrier function. For salicylic acid, the same dose acted as an infinite dose when using *ex vivo* models and synthetic membranes and as a finite dose when using reconstructed skin simulants. The human skin explants remained viable during dynamic culture, as shown by histopathological analysis.

Conclusions: The effectiveness of skin simulants in replicating human skin's barrier function depends on the compound being tested. Our skin-on-a-chip platform provides an efficient, multiplexed, and automated screening system that requires less material than traditional methods. With its ability to maintain a realistic microenvironment, it also enables long-term culture of skin samples, facilitating repeated dose and chronic studies.

Presentation: Poster

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Development of a robust multi-organ-chip system for human disease modelling

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Advances in microfluidic technology and 3D printing methods have enabled the design of physiologically representative microphysiological systems (MPS) that recapitulate complex human tissue microenvironments and have the potential to replace animal models.

The challenge of developing a dynamic, multiple organ-on-chip system requires an interdisciplinary approach and here we describe the development of a simple, robust microfluidic gut-lung-brain MPS. This modular system combines bespoke microfabrication of interconnected organ-chips with co-culture of differentiated human tissue models. The incorporation of microfluidics enables modelling of the peripheral blood circulation between organ compartments.

Our results support the use of the gut-lung-brain MPS for a wide range of studies and disease models. A key advantage of our larger-scale system is that it is compatible with use at high-containment and provides the opportunity to monitor infectious disease progression between organ compartments.

Using high-resolution imaging platforms, we demonstrate the functional effects on and trafficking between individual organ compartments following drug administration (e.g., MPP+ toxicity) or microbial infection (e.g., SARS-CoV-2 or bacterial extracellular vesicles (BEVs)).

Our findings underline the potential of our bespoke MPS for use in preclinical studies to further understand disease mechanisms or monitor the efficacy and toxicity of novel drug candidates. Fur-

thermore, the potential to incorporate patient-derived tissues supports a new generation of patient-specific disease models.

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Development of a real-time cellular barrier integrity monitoring system in PDMS-free lung-on-chip devices

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Weakened endothelial barrier integrity enhances vascular permeability which can evolve in cancer metastasis with subsequent poor prognosis [1]. Transepithelial electrical resistance (TEER) is a promising non-invasive epithelial-endothelial barrier integrity monitoring method for a real-time measurements in Organ-on-chip devices (OOC), thereby replacing current fluorescent dye snapshot readouts and removing experimental ambiguity [2]. However, lack of standardization, electrode polarization and measurement instability hinder the integration of the technique in OOCs [3].

In our work TEER electrodes were implemented in PDMS-free devices for lung cancer (LCoC) and normal lung (LoC) cellular barrier integrity monitoring in real-time. Devices and electrodes were fabricated by soft lithography and thermal evaporation, respectively. In order to ensure consistent conditions during measurement recording, a custom TEER measurement set-up was developed. To establish a measurement baseline and optimize the setup for noise and measurement stability, TEER values of various phosphate buffer saline concentrations were measured across different devices.

Post optimization, results showed only 0.03% variation in TEER values. To evaluate device performance, primary human small airway epithelial cells and primary human microvascular endothelial cells were cultured in a vertically stacked design. Cellular barrier formation was monitored throughout the culturing time and TEER values were cross correlated with 20 µg/mL Lucifer yellow permeability tests showing a good correspondence. After 2 weeks of LoC system being under air-liquid interface (ALI), cisplatin was added to the endothelial cell media at concentration 20 µg/mL and continuously flushed through endothelial channel at a flow rate of 2 µL/min for 48 h. Subsequent decrease in TEER was measured in the real-time setup.

In conclusion, a real-time TEER measurement system was integrated in PDMS-free LCoC and LoC model achieving ALI with lung epithelium on the top channel and pulmonary microvascular endothelial cells on the bottom. Tissue permeability plateau was confirmed after 2 weeks in ALI conditions in both – TEER and lucifer yellow permeability tests. Significant drop in TEER values was observed after cisplatin was introduced in the system, confirming the cellular barrier disruption.

References

[1] *Cancer Sci 112*, 2966-2974 (2021).
[2] *Lab Chip 19*, 452-463 (2019).
[3] *ACS Biomater Sci Eng 7*, 2926-2948 (2021).

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A new lung-on-chip platform for acute inhalation toxicity assessment and treatment

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Prolonged exposure to toxic inhalants expedites the development of chronic lung conditions like emphysema, a severe manifestation of chronic obstructive pulmonary disease (COPD). The pulmonary epithelium, specifically the alveolar-capillary barrier, serves as the main portal of entry to the systemic circuit for the inhaled irritants. Innovative "new-approach-methodologies" utilizing human-derived cells and tissue have the potential to overcome the limitations of animal studies in clinical translation.

Here, we have employed the novel Cloud α AX12 platform that seamlessly integrates the cutting-edge ^{AX}lung-on-chip (LOC) technology (AlveoliX AG) with a cloud-based exposure system (Vitrocell GmbH) to mimic realistic inhalation exposure in the distal lung. A triple co-culture system, comprising human alveolar epithelial cells, macrophages and endothelial cells, was established on-chip at air-liquid interface (ALI) with/without breathing-like (BR) stretch conditions. Environmental triggers were recreated by using either nanoparticles (NPs) like ZnO and TiO2 or chemicals like PHMG. To evaluate our model in a drug efficacy context, the corticosteroid fluticasone propionate (FL), was nebulized to mitigate PHMG-induced inflammation.

Comprehensively, nebulized ZnO and TiO2 NPs under ALI+BR conditions incited a pro-inflammatory cascade leading to disrupted alveolar barrier (transepithelial barrier resistance; TER decreased ~3 fold), increased cytotoxicity (~2.5 fold) and increased pro-in-

flammatory gene expression (IL6, IL1β, TNFα and ICAM1). Additionally, exposure to aerosolized PHMG resulted in significant cytotoxic effects, including barrier breakdown, epithelial-mesenchymal transition and elevated gene expression of inflammation-associated cytokines (IFNγ, IL6, IL8, IL1β and Arginase2) in mono- and triple-cell culture models. Furthermore, our results showed that nebulized FL effectively alleviated the toxic effects of PHMG, including EMT and inflammation. The Cloud α AX12 platform thus enabled us to establish distinct exposure models with varying cellular complexity, allowing us to gain insight into the crucial physiological impact of ALI+BR culture conditions, which made the alveolar barrier more sensitive to the uptake of aerosolized substances.

The Cloud α AX12 platform offers reproducible conditions and ease of use for inhaled medicine development and hazard assessments. Our results strongly support the use of this inhalation tool as an alternative to animal models in inhalation toxicity and drug efficacy testing, particularly in pre-clinical and precision medicine studies.

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Pan-cancer microfluidic platform for functional precision medicine aided by computer vision

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Functional precision medicine aims to select a beneficial treatment for a particular patient. Although the gold standard for pairing patient-treatment is genomic testing, clinical data suggests that genomics alone falls short in providing access to precision cancer medicine in the wider population. Advances in physiologically relevant 3D biomimetic environments have enabled the development of sophisticated platforms including more representative models to mimic cell niches, interactions and cross-talk communication allowing for the next generation of functional precision platforms [1].

Pear Bio combines patient tumour samples and matched blood, cell population labelling, proprietary hydrogel formulations, organ-on-chip platform, time-lapse confocal imaging and computer vision analyses to test a variety of approved chemo- and/or targeted therapeutics *ex vivo* for functional precision (immuno-)oncology [2]. The core of Pear Bio's technology is the patient: every patient is unique and thus responds differently to each treatment option. Pear Bio has developed robust primary cell extraction protocols and biomimetic hydrogel formulations to recreate 3D tumour microenvironment (TME) architectures of target tissues, which have been tested on 9 different solid tumour types. In addition, immunocompetent microenvironments have been developed by the addition of patient-derived peripheral blood mononuclear cells to allow tracking of immune-cancer interactions in the 3D TME. Fluorescently-labelled cells in a personalised TME are tracked over time using confocal microscopy and exposed to potential treatment options. The collected images are processed with our proprietary computer vision pipeline and analysed to obtain functional metrics such as cell viability, migration, immune cell infiltration and tumour architecture evolution over the treatment period. The metrics and daily images are compiled together with the demographics of the patient, to produce a report comparing relative treatment efficacies. Pear Bio's early results have shown > 80% sensitivity and specificity metrics when retrospectively compared with real-world patient outcomes in triple-negative breast cancer. Currently, 3 observational clinical trials aim to determine sensitivity and specificity in a prospective manner. In summary, the Pear Bio workflow and report would enable oncologists to make more informed decisions regarding treatment options.

References

[1] Letai, A. et al. (2022). *Cancer Cell 40*, 26-35.
[2] Ranatunga, D. et al. PAP US20230008421A1

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Unleashing the intravasation potency of non-stem-like lung cancer cells with EMT features: A functional microvasculature approach

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Introduction: The prediction of tumor progression, especially the likelihood of distant metastasis, is a critical challenge in oncology. Metastasis, the spread of cancer cells from one part of the body to another, is the most common cause of death in cancer patients. Despite its importance, the mechanisms of metastasis are still not well understood, mainly due to the difficulties in studying this complex

process. To gain a more profound understanding of the mechanisms of metastasis, advanced *in vitro* models mimicking the complexity of tumor microenvironments are needed to accurately simulate the intravasation and extravasation of tumor cells – two critical steps in the metastatic process.

Methods: In response to these challenges, we developed a new functional microvasculature system designed to closely mimic the tumor microenvironment and simulate cancer cells' intravasation and extravasation *in vitro*. In this study, non-stem-like lung cancer cells with epithelial-mesenchymal transition (EMT) features were seeded on top of the microvasculature bed. The system was then incubated to allow the metastasis journey of the tumor cells. The intravasation potency of the cancer cells was then studied by analyzing their ability to penetrate the vessel walls before entering the microvasculature network.

Results and discussions: This study showed that the non-stem-like lung cancer cells with EMT features could intravasate into the microvasculature and extravasate from the microvasculature to a secondary site. These results demonstrate that functional microvasculature models, such as the one developed in this study, have the potential to improve our understanding of the complex process of metastasis. By accurately simulating the intravasation and extravasation of cancer cells, these models can provide new insights into the mechanisms of cancer progression and help identify new targets for the prevention and treatment of metastasis.

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A breast cancer derived organoid model reveals an unlikely event: How *in vitro* data can inform *in vivo* tumour behaviour

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The current breast cancer treatment regimens available to South African patients are largely informed by data gathered from studies performed in Europe and America.

Background: Variability in treatment responses and adverse effect profiles have been reported, necessitating the need for establishment of a breast cancer cell model that better represents the African population. Over the last two year, we have collected, established and expanded breast cancer organoid lines derived from tissue from black African women treated within the South African public health system. Here we show data on one line isolated with a rare phenotype *in vitro* that explains its behaviour *in vivo*.

Materials and methods: A patient undergoing a mastectomy was asked to participate in our organoid biobank study. After consent, a small part of the tumour was resected after surgery and used to establish an organoid line. The line was assessed for adhesion to different substrates, and confocal imaging was used to assess cell adhesion protein expression and localisation. Adhesive capacity was further quantified by binding assays to fluorescent collagen probes. Genomic analysis was performed to identify somatic cancer gene mutations.

Results: The organoid line isolated from this patient was unique in that it were not able to adhere and spread on cell culture plastic, collagen, BME or fibronectin. In contrast, a number of other lines tested were able to grow and migrate in 2D even on plastic. A collagen probe-binding assay was done to assess binding capacity further. The patient presents with large stage III T3N1M0 tumours.

Conclusion: Our initial results show that a proliferative but non-metastatic breast tumour generated breast cancer organoids that had a complete lack of cell-substrate adhesion even though cell-cell adhesion was functional and robust. Cells lacked the ability to attach and spread on surfaces including collagen, fibronectin and even BME, correlating with the observation that even with large tumours no metastasis was observed in the patient.

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An innovative three-channel micro cavity-equipped microfluidic biochip to generate patient-derived pancreatic cancer spheroid-on-a-chip models for screening novel therapeutic approaches

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Pancreatic ductal adenocarcinoma (PDAC), the most common pancreatic cancer subtype, is among the deadliest solid malignancies with a five-year survival rate of less than 10%. Due to the high unmet medical need, the development of novel, ideally targeted therapies are urgently needed. The characteristic immunosuppressive tumor microenvironment (TME) with an extensive desmoplastic stroma plays a major role in PDAC growth, invasion, and metastasis. However, while failing to recapitulate the cellular heterogeneity and structural complexity of 3D PDAC tumors, to date, most *in vitro* and *in vivo* PDAC models available have limited translation relevance with respect to the clinic. The animal studies in pancreatic cancer research are often using immunocompromised mice, which cannot develop the microenvironment typically

found in human pancreatic carcinoma. In addition, mouse tumor cells lack many of the gene mutations typical of human pancreatic carcinoma.

Thus, we here describe the development, validation, and importance of a novel TME *in vitro* model for PDAC to improve the therapeutic approaches. We established a microfluidically supported human 3D PDAC co-culture model in a new and innovative three vertical chambered biochip. The platform supports on-chip 3D spheroid culture in specifically designed porous microcavities and provides means for integration of vascular components. Hence, a continuous physiological nutrient supply for the micro-tumors can be simulated. The 3D PDAC micro-tumors consist of pancreatic tumor cells and primary patient-derived cancer-associated fibroblasts supporting the characteristic stiff and immunosuppressive microenvironment of this type of cancer. We were able to show that the spheroids are able to withstand the physiologically relevant shear forces and flow rates in the biochip. Cellular distribution in the 3D spheroid has been shown to be maintained. Furthermore, a significantly improved spheroid vitality is achieved by continuous nutrient supply via double perfusion for up to 48 h than in static control groups, which was expressed in improved marker expression.

Next steps include the integration of important immune cell types such as tumor-associated macrophages, which is currently investigated, and different kind of T cells. Our 3D tumor spheroid-on-chip model enables phenotypic drug screening and, prospectively, immunotherapy research for PDAC.

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Chances and challenges for *in vitro* models to address CNS toxicities

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The development of novel drugs for CNS disorders is becoming a growing unmet medical need. Successful development not only relies on the identification of novel, promising targets but also on the application of novel therapeutic modalities beyond small molecules. To cope with the development of new modalities also safety assessment needs to be adapted. This comes along with multiple opportunities for the application of *in vitro* models to address safety aspects.

What risks need to be addressed by safety assessment and where are the opportunities for *in vitro* models? In the field of ophthalmology one potential risk of antibody-based drugs targeting retinal diseases are intra-ocular inflammations potentially leading to sight threatening side effects. Immune-competent *in vitro* models of the retina and/or vasculature could help to better understand mechanisms in the clinic leading to development of molecules devoid of certain risk factors or to the stratification of patient populations. Novel gene therapy assets for the retina could as well trigger inflammatory related side effects. Those could be addressed using immune-competent models of the retina. Furthermore, for ocular disorders affecting the front-of-the-eye, such as the cornea, early safety testing must aim at avoiding compounds and formulations triggering corneal irritation. In the brain, novel modalities such as mRNA therapeutics bear the risk of activating the adaptive and innate immune system leading to subsequent effects in other cell populations or might have a direct effect on neurons. Immune-competent models of the brain could help to further derisk and uncover potential mechanisms supporting best selection of drug candidates. We will highlight examples from early drug development showcasing opportunities as well as gaps of *in vitro* models to address the needs for safety assessments.

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Vascularization and cellular rearrangement in bioactivated gellan gum hydrogels

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Vascularization of different tissue models is one of the main targets in Organ-on-Chip (OoC) technology. Recapitulation of blood vessel network *in vitro* gives substantial benefits including mechanical support, angiogenic cytokines, maturation cues and a transport system for oxygen delivery and immune responses. Human adipose stem cell derived stem/stromal (ASCs) cells are known to support self-assembly of endothelial cells into perfusable vascular network [1]. Moreover, hydrogels are an attractive tool to develop OoCs with 3D environment, as they permit diffusion and support 3D cellular organization. In this study, we studied cellular rearrangement and vascularization in four different hydrogels combined of gellan gum (GG) and gelatin [2].

The four different hydrogel modifications included (1) purified NaGG; (2) avidin-modified NaGG combined with biotinylated fibronectin (NaGG-avd); oxidized GG (GGox) covalently modified with (3) carbohydrazide-modified gelatin (gelaCDH) or (4) adipic hydrazide-modified gelatin (gelaADH). ASC (1 x 10^6 cells/ml) and GFP tagged Human Umbilical Vein Endothelial Cells (GFP-HUVEC, 5 x 10^6 cells/ml) were encapsulated in the hydrogels and cultured for 14 days in angiogenic EGM-2 medium

(Lonza). Cellular reorganization and vascularization were analyzed following the GFP signal from HUVECs and with immunocytochemical staining using α-smooth muscle actin (αSMA) antibody with confocal imaging, OPT and SPIM.

GG-based hydrogels were chosen due to their capability to provide mechanical strength and gelatin for proving cell attachment sites in the hydrogel matrix. Microscopical analyses revealed that in NaGG and NaGG-avd, cells remain rounded and do not organize into structures. In gelaCDH and gelaADH hydrogels, short vascular structures can be detected. Additionally, gelaCDH and gelaADH support ASC organization into network stained positive with αSMA and its partial alignment with vascular structures. Gradual degradation of the cell-free hydrogels can be detected during the 14 day culture.

We showed that the gela(CDH)-GG(A) hydrogel formulation supports simultaneous formation of vascular structures and smooth muscle cell-like network. The ability to stimulate cellular reorganization and vascularization with different hydrogel scaffolds is an important step towards personalized OoCs.

References

[1] Mykuliak, Yrjänäinen et al. (2022). *Front Bioeng Biotechnol 10*, 764237. doi:10.3389/fbioe.2022.764237

[2] Gering et al. (2022). *Biomater Adv 143*, 213185. doi:10.1016/j.bioadv.2022.213185

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Multi-organ-on-chip to study breast cancer metastasis

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Billions of dollars are spent on new cancer drug development, yet effective treatment for the disease remains elusive. The FDA approval rates for a new drug from phase 1 to approval stand at an abysmal 3.3%, indicating that for every hundred drugs developed, 97 do not materialize. This can be attributed to a lack of a proper model to predict human drug response. Although capturing essential information, typical monolayer cell cultures on plastic dishes do not recapitulate the physiological 3-dimensional microenvironment, while the current animal models lack human-specific predictions. Organ-on-chips are a class of engineered miniaturized models to help bridge the gap. We have initiated the design, fabrication, and validation of multi-organ-on-chip (MoC) to mimic the breast cancer microenvironment along with other relevant organs for metastasis. The design includes multiple organ spaces to simultaneously culture cells of different organ sites. A unique design feature allows us to control the seeding and maintenance of cells of different origins separately to cater to specific needs such as extracellular matrix, shear rate, and growth factors. A common vasculature-mimicking circuit connects all the organ spaces and mimics the essential mechanical forces faced by cancer cells in circulation. The organ spaces and the vasculature are sandwiched with a semi-permeable membrane in between to facilitate the crosstalk between the cells in circulation and the organs. As a start, we have initiated culture of MDA-MB-231 (Breast cancer) and A549 (Lung) in the device as a proof of concept. The live cells in both organ spaces were imaged by labeling them with Calcein AM (Live marker) and Cell Tracker (Nucleus). The cells were imaged after 48 h of culture in the MoC. In the future, we envisage functionally mimicking the breast tumor environment coupled with other organs to model disease conditions and probing for several markers to understand the metastatic cascade to develop novel cancer targets.

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Muscle microphysiological system to model tissue degradation: Opportunities to study the impact of microgravity and accelerate drug development

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Muscle degradation leads to a dramatic decline in mobility in the aging population, yet age-related muscle atrophy or sarcopenia remains without effective therapeutic options, largely because molecular changes accumulate over decades. Skeletal muscle microphysiological systems or tissue chips have the potential to advance screening of drugs to counteract pathologies *in vitro* with clinically relevant physiology. Tissue chips are adaptable to space flight for monitoring the effects of microgravity which may mimic the salient features of sarcopenia in a faster timescale. Monitoring contractile function allows for real-time data collection and analysis of microstructural changes that underlie muscle degradation. We have made advancements in the development of an autonomous human muscle-on-chip CubeLab that incorporates biopsy cells isolated from young and older volunteers into 3D myobun-

dles encapsulated into a microfluidic chip. On SpaceX CRS-25, we captured, real-time contractile function of the human myobundles on-orbit in the International Space Station (ISS) and on SpaceX CRS-26, we evaluated the anti-atrophic natural product, tomatidine. In ground studies pre-flight, we have shown improvement in contractile function in myobundles derived from older donors treated with tomatidine and we have analyzed multiple omics studies comparing young and old derived tissue chips to detect relationships between gene pathways and unravel new targets for countermeasure development.

In this work, we present our data on tomatidine treatment. We will also describe our results from digital image correlation analysis, processed in HiPerGator supercomputing system, to generate displacement graphs and analyze magnitude displacement differences between young and old derived myobundles treated on ground and in space. We describe our approach to extract microscopic data related to homogeneity, synchronicity, and uniformity of myobundles analyzed at 40 fps over successive days for development of a model for muscle degradation that incorporates the difference between extrinsic (force generating) and intrinsic (elasticity) muscle properties. Overall, our tissue chip payload enables functional monitoring of donor-derived myobundles with electrical stimulation with the longer-term goals to identify effective exercise mimicking regimes, evaluate therapeutic compounds to benefit muscle health and establish a MPS precision medicine approach on ground and in space for therapeutic development to treat sarcopenia.

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Automated platform for the micro-perfusion of bioengineered tissues

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Oxygen and nutrient delivery are vital in tissue engineering. Perfusion systems aimed at mimicking *in-vivo* conditions are essential to recreating a physiological micro-environment for *in vitro* tissue maturation. Unidirectional and continuous medium flow through the engineered tissue is required to provide oxygen and encourage the growth of a vascularized network. Proposing a disruptive alternative to donor organs for liver regenerative medicine, the EU project ORGANTRANS is developing a liver tissue printing platform from cell source to functional tissue, relying on organoid technology as building block for liver tissue transplantation [1].

In this context, a fluidic platform was developed to perfuse printed liver bioconstructs, while supporting standardization. The system is compatible with multi-well plates and relies on a microfluidic "Smart Lid" for perfusion, leading to automated and parallelized medium circulation, enabling long-term tissue growth vascularization and maturation. The perfusion platform consists of six disposable sterile inserts, into which the bioconstruct is printed, and that fit into a standard 6-well plate; a sterile lid with integrated microfluidic features, sealed onto the inserts and creating a closed perfusion chamber; a multi-channel peristaltic pump with fluidic tubing and connectors; as well as electronic supply and control. Biocompatible and sterilizable materials are used for all parts that are in contact with the tissue and cell culture media for biocompatibility.

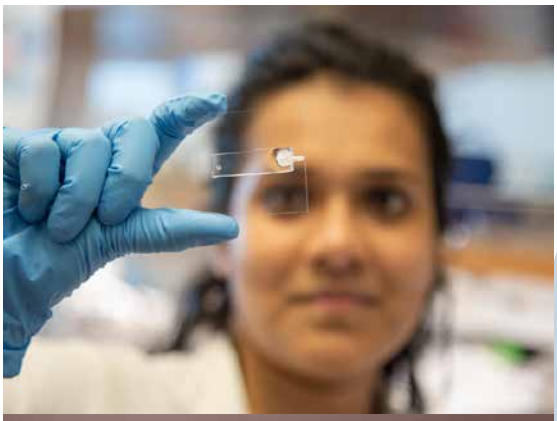
The perfusion platform enables continuous and unidirectional flow through the six wells in parallel, with tunable flow rates ranging from 10 to 500 µL/min without any leakage. Perfusion of 2D cell culture was demonstrated for two weeks in an incubator, showing cell proliferation and no impact on cell viability. Continuous perfusion through six 1 x 1 x 0.5 cm^3 freshly printed spheroid-laden liver bioconstructs containing channels lined with endothelium will be presented.

The perfusion platform is used to test and tune the maturation process of liver tissue bioconstructs for several weeks, promoting vascularization while ensuring a sterile and closed environment. The platform can be adapted to other tissues and geometries, thereby enabling standardization of tissue engineering processes for regenerative medicine and drug testing applications.

Reference

[1] ORGANTRANS project, EU Horizon 2020 research and innovation programme (grant agreement No 874586). www.organtrans.eu

Presentation: Oral



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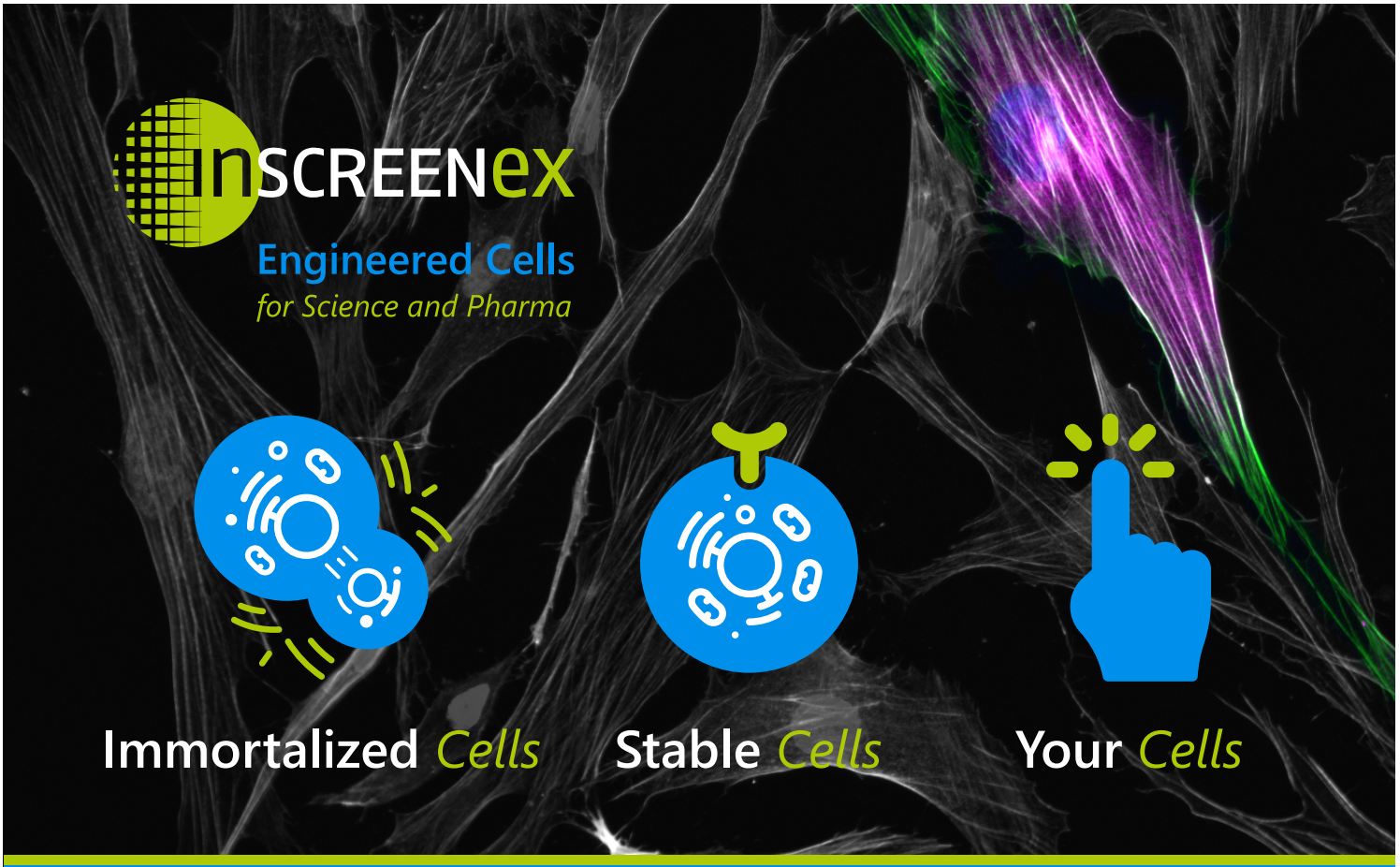

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A representative full thickness skin model with optimised geometry simulating the dermis and epidermis

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Biomedical research is always closely related to clinical trials, which are a major financial burden for investors. Therefore, the idea of *in vitro* models, in which newly developed drugs, cosmetics and medical devices can be tested in a representative way, thus reducing animal testing and clinical trials to a minimum, is one of the most sought-after research approaches. Testing substances with complex full-thickness (FT) skin models offers several advantages over animal testing and experiments with human subjects [1]. The results obtained are reproducible, and testing can be performed more effectively and quickly. The construction of a FT skin model consisting of an epidermal layer with keratinocytes and a dermal layer with fibroblasts represents a foundation for developing a complex engineered skin model (encompassing epidermal, dermal, and hypodermal layers with representative human cells and blood vessels). By combining 3D bioprinting technology and cell culture techniques, we designed a 3D bioprinted, human cell-based skin model [2] with anatomically relevant structural and mechanical features that resemble human skin.

Cellular and histological hallmarks regarding long-term cell viability, deposition of extracellular matrix (ECM) components, as well as differentiation and keratinisation markers were analysed. The 3D polysaccharide-based microenvironment supported the proliferation and differentiation of embedded human skin fibroblasts. Moreover, it engaged the deposition of ECM proteins, which are important for skin development and ECM organisation. The attachment and migration of keratinocytes on the cell construct promoted differentiation to form the epidermis. Such 3D bioprinted constructs have the potential to be highly applicable for topical drug or cosmetic testing compared to conventional trans well cultures and animal models.

The authors acknowledge the financial support from the Slovenian Research Agency for Research Core Funding No. P3-0036, and for projects Nos. J3-1762, J7-4492, and L7-4494.

References

[1] Zidarič, T. et al. (2023). Function-oriented bioengineered skin equivalents: continuous development towards complete skin replication.

[2] Zidarič, T et al. (2020). Polysaccharide-based bioink formulation for 3D bioprinting of an in vitro model of the human dermis. *Nanomaterials 10.4*. 733.

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3D-bioprinting of bacterial biofilm on monolayer of human lung cells as advanced *in vitro* model for chronic lung infections

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Biofilm-forming bacteria can be up to 1000-fold more tolerable to antibiotic treatments than their planktonic counterparts [1], which necessitates the urge to find novel therapeutic approaches. Novel developed drug formulations must be tested for safety and efficacy before considering subsequent clinical studies. The current standard is to test them on animal models which are, however, ethically problematic and moreover limited by major differences to humans. Current *in vitro* models of bacterial biofilms do not allow addressing any host-pathogen interactions or show drawbacks in terms of reproducibility and design.

Expanding on recently reported pipetting of biofilm microclusters [2], we here report a novel approach using 3D-bioprinting to generate an improved human-based *in vitro* infection model. Essentially, pre-formed mature biofilms are printed on confluent layers of human epithelial cells grown at Air-liquid-Interface. First, a gelatin-alginate based bioink was developed to print *E. coli* MG1655 biofilms on human bronchial epithelial cells (Calu-3). Biofilm properties were maintained after printing as shown via different assays.

Secondly, we adapted the technique to print biofilms of *P. aeruginosa*, the main pathogen inhabiting the lungs of cystic fibrosis patients [3], on these cells to mimic chronic lung infections. For that, an agarose-alginate bioink proved to be suitable for its cultivation and biofilm formation. The 3D-printed biofilm exhibited biofilm properties. After adjusting the printing parameters, thin layers of the blank bioink were printed on epithelial cells to exclude any negative impact on cell viability and barrier integrity. Furthermore, transport studies were carried out to ensure that the established bioink does not impair drug permeation.

Complex *in vitro* models comprising bacteria and human cells may allow generating readouts for both the pathogens (e.g., killing, virulence factors) as well as the epithelial cells (e.g., vitality, barrier properties) in parallel. The model is currently being further validated

as a potential alternative to animal experiments for preclinical testing of novel anti-infectives.

The SET-Foundation (Germany-Frankfurt) is thanked for financial support.

References

[1] Wagner and Iglewski (2008). *Clin Rev Allergy Immunol 35*, 124-134.
[2] Horstmann et al. (2022). *ACS Infect Dis 8*,137-149.
[3] Lyczak, Cannon and Pier (2002). *Clin Microbiol Rev 15*, 194-222.

Presentation: Oral

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Flow affects orientation of iPSC-derived vascular smooth muscle cells in a patient-specific 3D blood vessel model mimicking *in vivo* morphology and pharmacological functioning of arteries

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Blood vessels can constrict or relax in response to vasoactive substances, thereby controlling blood flow to different parts of the body. Here, we developed a 3D patient-specific blood vessel model using induced pluripotent stem cells (IPSCs), in which the orientation of IPSC-derived vascular smooth muscle cells (VSMCs) mimics the architecture of functional blood vessels *in vivo*. VSMCs should align perpendicular to the flow to allow narrowing and widening of the artery and the cultured blood vessels should be able to functionally respond to vasoactive substances.

IPSC-derived VSMCs were seeded in a tubular collagen structure inside a PDMS chip consisting of a round channel and exposed to low flow (shear stress 0.5-0.8 dyn/cm^2) for 3-5 days. Local gradient orientation analysis revealed that more VSMCs aligned perpendicular to the channel after exposure to flow, resulting in 53% of the image having the desired orientation (defined as an orientation between -30° and 30°, with 0° being perpendicular to the channel), compared to 28% of the image in the absence of flow. Moreover, the cultured blood vessels respond to vasoactive substances, resulting in increases in intracellular cAMP (cADDis live cell cAMP assay), as a proxy for vasorelaxation, in response to vasodilators CGRP, adrenomedullin and adrenomedullin 2 and increases in intracellular Ca^{2+} (calcium dye Cal-520) as a proxy for vasoconstriction, in response to vasoconstrictors endothelin-1 and angiotensin II. Additionally, pharmacological intervention using receptor antagonists or enzyme inhibitors successfully affects these vasoactive responses of 3D cultured blood vessels. Responses in the 3D blood vessels were compared with responses in human isolated coronary arteries, as measured in a Mulvany myograph system, and were shown to be in the same order of potency, indicating the vascular receptor expression and function is similar in human coronary arteries and the cultured blood vessel.

Application of flow results in an improved 3D vessel-on-chip model. Future research should focus on including IPSC-derived endothelial cells, which should align in the direction of the flow to form a tight barrier, and on optimizing the flow rate and duration in order to create a blood vessel resembling the *in vivo* architecture as accurately as possible.

Presentation: Poster

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Design of a bioprinted microfluidic chip as a tumor liver model for drug screening

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Introduction: Drug discovery is still a risky, expensive, and time-consuming process. The preclinical research phase relies mainly on the use of *in vitro* and *in vivo* models that even when combined, do not provide a perfect representation of the human physiology and function.

Microfluidic chips are a promising tool to study complex biological systems, such as drug efficacy and toxicity, by recapitulating the liver 3D microenvironment. In this case, a microfluidic chip was created by combining 3D bioprinting and a sacrificial template method [1]. Hence, a polydimethylsiloxane (PDMS) microfluidic chip was produced, designed to mimic one sixth of a hepatic lobule, to study the interaction between endothelial cells and tumor liver spheroids for drug screening.

Methods: A Bioprinter (BioScaffolder, GeSiM) was used to print a sacrificial material (thermo-responsive Pluronic F-127), according to a pre-designed G-code. Pluronic F-127 40% (w/v) was bioprinted on top of a cured PDMS layer and partially covered by PDMS after bioprinting. After curing, the chip was covered with PBS and placed at 4ºC for 48 h to remove the sacrificial material. Following sterilization, the PDMS chip was coated and HUVECs

seeded to form an endothelial cell monolayer. In parallel, HepG2 spheroids were produced in an *ad-hoc* PDMS platform and when the HUVECs reached confluence inside the chip, HepG2 spheroids were transferred to the respective chip cavities.

Results: A reproducible microchip production was obtained by tuning the microchip design and bioprinting parameters. HUVECs successfully formed a confluent monolayer 48 h after seeding. The tumor spheroid model was inserted in the chip and its interaction with HUVEC monolayer was assessed.

Conclusions: We were able to design and produce a simple microfluidic chip that can be used to study the interaction between endothelial cells and a tumor model, better resembling the physiological environment than monoculture systems. This microfluidic chip will be used as a relevant system to assess drug efficacy and toxicity.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860715.

Reference

[1] Ji, S., Almeida, E. and Guvendiren, M. (2019). *Acta Biomater 95*, 214-224.

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Multi-axis MPS enabling *in vitro* tissue mechanical stimulation for musculoskeletal research

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Low back pain is a worldwide social and economic burden and appears in the top five causes of disability-adjusted life year worldwide [1], reducing the ability to work and perform daily tasks. In recent years, progress has been made regarding potential therapeutics that could have restorative actions targeting intervertebral discs (IVDs). However, those developments are impeded by the lack of an *in vitro* model for drug assessment that can mimic typical motions occurring in the human body while maintaining IVD viability.

In this work, we present a unique automated micro-physiological system (MPS) that enables six degrees of freedom (DOF) actuation on an *ex vivo* bovine IVD organ model in a controlled environment. This MPS efficiently transfers forces to the IVD, mimicking flexion, extension, lateral bending, torsion, tension, and compression in confined and sterile conditions. It consists of a biochamber accommodating the IVD specimen using customized sample holders; fluidic ports to enable automated medium exchange; a hexapod as the actuation platform with an integrated force sensor; and software enabling the control of the entire platform.

This advanced MPS enables mechanical actuation from 1- to 6-DOFs under sterile conditions, showing no contamination after 9 days. The newly established IVD organ model for multiaxial bioreactors maintains cell viability to a similar level as the IVD model commonly used in the uniaxial bioreactors (outer and inner annulus fibrosus 94%, nucleus pulposus 84%), thus confirming its capacity for long-term biological studies under mechanical loading. In addition, mechanical tests showed that the IVD-holder interface can sustain compression forces above 200 N, torsion above 1.3 Nm, bending higher than 0.3 Nm, and tension around 100 N, which is higher than the reference degenerative values for bovine IVD [2].

By replicating specific spine motions, this advanced MPS enables the development of advanced *in vitro* organ degeneration models for musculoskeletal research and drug and treatment studies.

References

[1] GBD 2019 Diseases and Injuries Collaborators (2020). Global burden of 369 diseases and injuries, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet 396*, 1204-1222.

[2] Šećerović, A. et al. (2022). *ACS Biomater Sci Eng 8*, 3969-3976.

Presentation: Poster

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Studying liver-islet crosstalk in a microphysiological system under healthy and diseased conditions

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There is a close link between metabolic liver diseases, type 2 diabetes, and obesity resulting in a dysregulation of glucose and lipid metabolism. Understanding the mechanism of inter-organ crosstalk and how it relates to metabolic homeostasis, offers a great potential to identify novel therapies. Conventional cell culture models lack complex multi-tissue interactions found in the human body and are unable for accurate disease modeling and predictive drug testing *in vitro*. Here, we have combined 3D human liver and islet models in a microfluidic multi-tissue system and characterized tissue crosstalk under healthy and diseased conditions.

3D human islet microtissues (hIsMTs) and 3D human liver microtissues (hLiMTs) were cultured at defined number ratios in the

Akura™ Flow MPS for 7 days. Each unit consists of 10 compartments, which are interconnected by a straight channel with a medium reservoir on each side. Repeated tilting of the plate leads to gravity-driven flow from the upper reservoir along the channel to the lower reservoir and thereby enables continuous perfusion and interconnection of the microtissues.

First, we established an insulin-free co-culture medium and by varying glucose and free fatty acid (FFA) concentrations, we could either preserve the healthy state or induce different metabolic disease phenotypes in the liver and islet models.

The crosstalk was demonstrated by the response of hLiMTs, metabolising glucose into triglyceride (TG) and glycogen, in dependence of insulin secretion by hIsMTs. High glucose ± FFA led to hyperinsulinemia, measured by chronic insulin secretion over time. Elevated levels of insulin resulted in steatosis induction in the hLiMTs, measured by elevated level of TG and glycogen. Additionally, glucose stimulated insulin secretion (GSIS) showed stages of beta cell failure, a hallmark in the progression of T2D.

Our Akura Flow system recapitulates the major endocrine hallmarks of liver-islet crosstalk in a highly scalable format. This work completes the next step towards its application as a drug discovery tool to identify anti-steatotic and anti-diabetic drugs.

Presentation: Poster

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Development of a high-throughput, 3D spheroid co-culturing platform for investigation of tissue interactions

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In vitro systems for studying human tissue interactions faces severe challenges regarding through-put, reproducibility, compatibility with multiple devices and endpoints, and general ease of operation. Implementation of advanced human 3D models in the drug discovery pipelines might reduce the use of frequently semi-optimal animal models and facilitate the development of new clinical candidates.

To address these limitations, we developed a high-throughput, 384-well-format platform for co-culturing two or more 3D spheroid models side-by-side, which is compatible with vast majority of laboratory devices and bioassays.

As a proof of concept, we performed a combined assessment of anti-tumor efficacy and liver safety of bispecific antibodies. We employed 3D spheroids composed of primary human hepatocytes and Kupffer cells as liver model. The solid tumor model was aggerated from a human cancer cell line (HCT116-GFP) and primary cancer associated fibroblasts, mimicking the tumor microenvironment. Both models were cultured in individual wells pairwise interconnected with microfluidic channels. Gravity-driven tubeless flow ensured tissue-tissue interaction.

To evaluate our system, we treated 3D spheroid models with runimotamab (HER2xCD3 bispecific antibody) in the presence of peripheral blood mononuclear cells (PBMCs). Tumor viability and growth was assessed by fluorescence measurements, while liver toxicity and function were monitored by release of liver aminotransferases ALT/AST, albumin secretion and live confocal microscopy. Immune cell activation was assessed by a cytokine bead array.

The treatment with runimotamab resulted in a significant decrease of fluorescence and size of tumor spheroids. At the same time, we observed an induced secretion of cytokines with a peak of expression of IL-2, TNFα after 24 h and INFy, IL-6 and IL-17A after 48 h. Cytokine release was coupled with elevated levels of clinically relevant liver damage biomarkers ALT and AST with peak at 72 h timepoint. This data suggests potential risk of cytokine release syndrome (CRS) followed by liver damage. Interestingly, redosing of the antibody was not followed by another release of liver enzymes, similarly to clinical observations.

In summary, we have developed a high-throughput platform for the investigation of complex tissue interactions. Automation-compatible with up to 192 co-culture conditions per plate it represents a powerful tool for drug discovery.

Presentation: Oral

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Efficacy assessment of novel anti-OA therapeutic drug candidates within an advanced mechanically active osteoarthritis-on-chip model: The SYN321 case study

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With the aim to reduce osteoarthritis (OA) pain and inflammation states, the development of polymer-drug conjugates is becoming a key factor to ensure a sustained release of non-steroidal drugs upon intra-articular injection (Kawanami et al., 2020). In this scenario, Organs-on-Chip enable to test the efficacy of such therapeutic products in human physiologically relevant *in vitro* models, by integrating key elements of native microenvironment. The aim of the presented work is the qualification of a novel microfluidic platform

able to reproduce 3D human OA microtissues (i.e., uKnee model) with a novel therapeutic drug candidate based on diclofenac linked to a modified sodium hyaluronate (NaHa) backbone (i.e., SYN321) (Rhodin et al., 2021).

The microfluidic platform allows the generation of mechanically-induced OA microtissues thanks to uBeat® technology. A dedicated channel allows for injection of the therapeutic formulation, which is then cultured in contact with the OA microtissues and subjected to the same mechanical stimulation, resembling the *in vivo* environment.

Human articular Chondrocytes were embedded in a fibrin gel, injected in the platform and cultured for 14 days in chondrogenic medium to generate a 3D healthy cartilage construct. Then a hyper-physiological compression (HPC) was applied for 7 days to trigger the shift of the healthy microtissues towards OA phenotype (Occhetta et al., 2019) as confirmed by the significant ($P < 0.01$) upregulation of degrading (MMP13), pro-inflammatory (IL6, COX-2) and calcification-related genes (COL10A1). At the protein level, Aggrecan expression, index of matrix deposition, was lower compared to healthy controls, whereas MMP13 expression was higher.

SYN321 was then injected in the platform and cultured for three days under HPC. SYN321 showed an anti-inflammatory effect by decreasing gene expression of TNF-α, COX-2 and by significantly ($P < 0.05$) reducing IL6 compared to OA control. Notably, the downregulation of these genes was less marked in the positive controls (i.e., NaHA, Diclofenac). In addition, SYN321 proved to reduce matrix-degradation, since MMP13 expression decreased both at gene and protein level, compared to OA control.

In summary, the platform was successfully qualified with the novel therapeutic drug candidate SYN321, elucidating its beneficial effect in reducing OA traits in a native-like mechanically active *in vitro* model.

Presentation: Oral

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Development of an instrumented microfluidic system to study chemoresistance in pancreatic ductal adenocarcinoma

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Novelty: This research presents a model of pancreatic ductal adenocarcinoma (PDAC) using a co-culture of fibroblasts and cancer cells in a viscoelastic biomimetic matrix composed of collagen 1 and hyaluronic acid with alterable stiffness. The channel height of the system can be modified to mimic the intratumoral pressure constraint. Additionally, it features a measurement system based on impedance spectroscopy (IS) to allow non-invasive continuous monitoring of the culture.

Background: PDAC is characterized by a dense stroma composed of a large population of fibroblasts overexpressing ECM proteins [1]. High rigidity of the tissue and high internal pressure lead to deteriorated mass transport and low effectiveness of chemotherapy [2]. Current 3D static models do not predict this deteriorated mass transport [3]. Microfluidic systems have shown to recreate more representative conditions of the PDAC microenvironment [4]. IS has been shown to quantify chemoresistance studies, however without study of rigidity and solid stress effects [5].

Results: The PDMS microsystem has two parts: a base bonded to a glass substrate that serves as the channel, and a top part that closes the system and compress the microtissue. A micropositioning system makes it possible to know precisely the internal pressure. The electrode pattern (titanium/platinum bilayer) was optimized using COMSOL Multiphysics and is connected via a sliding connector to an impedance meter (Sciospec GmbH). The device includes a synthetic matrix whose porosity and permeability allow direct perfusion for several days without deterioration. Its composition can be changed to obtain different stiffnesses (1;8;16 kPa) and its mechanical properties are measured by compression test. Our culture conditions enable the expression of specific epithelial (E-cadherin) and mesenchymal (vimentin) markers, tumorigenic signalling pathways (β-catenin) and mechanobiological actors (YAP/TAZ hippo pathway) either with patient-derived tumoroids from pancreatic tail PDAC or co-culture of human pancreatic cancer cell lines (Capan-2/MiaPaCa-2) with activated human pancre-

atic stellate cells. Different chemical compositions were tested to optimize PS-1 activation.

References

[1] Nieskoski (2017). *Sci Rep*.
[2] Jain (2014). *Annu Rev Biomed Eng*.
[3] Haque (2022). *Microsyst Nanoeng*.
[4] Beer (2017). *Sci Rep*.
[5] Poenick (2014). *Biosens Biolectron*.

Presentation: Poster

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Predicting immune-related antibody-induced toxicities with microphysiological organ-on-chip models

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Introduction: Immunomodulatory biologics gained high significance as anti-cancer therapies in the past. Common therapeutic antibodies directly address tumor-associated targets on cancer cells to induce antibody-dependent cytotoxicity [1]. However, such antibodies can also cause harmful side effects to humans. The immunomodulatory agonistic antibody TGN1412, which targets CD28 on T cells, was intended for the treatment of a number of diseases such as leukemia and multiple sclerosis. The first-in-human trial was terminated after all six volunteers experienced a life-threatening cytokine storm with mainly elevated IL-2 levels [2]. Preclinical studies confirmed an effective role of the antibody, but without revealing any adverse response. To overcome the limitations of animal models or 2D cell cultures, we investigated a 3D microphysiological model of the human blood vessel to recapitulate antibody-dependent toxicities and immune-related adverse events (irAE).

Methods: The human blood vessel model, which incorporated either human umbilical vein endothelial cells or aortic endothelial cells, was assembled within the Dynamic42 biochip. CD4+ effector memory T cells were freshly isolated from human peripheral blood and analysed for CD4 via FACS staining. TGN1412 and CD4+ T cells were simultaneously perfused in the system for 24 hours. The emerging cytokine storm and its effects on endothelial cells were validated by cytokine analyses, permeability assay and immunofluorescence staining of junctional proteins.

Results and outlook: We could identify a strong release of IL-2 from CD4+ T cells that was induced by TGN1412 administration. Individually applied IL-2 could show the cytokine contribution to barrier disruption, including the breakdown of adherens junctions. These effects were determined by means of increased permeability to FITC-Dextran and diminished VE-Cadherin presence.

These findings contribute to the understanding and prediction of drug-induced toxicities of therapeutic antibodies such as cytokine release syndrome and vascular leakage. Our microphysiological model shows great potential to recapitulate these events occurred *in vivo* and could serve to overcome limitations of cell culture and animal models.

References

[1] Lu, R.-M. et al. (2020). *J Biomed Sci 27*, 1.
[2] Stebbings, R. et al. (2007). *J Immunol 179*, 3325-3331.

Presentation: Oral

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Optimisation of 3D thyroid and liver models for cross-species comparison of thyroid toxicity mechanisms

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Chemicals are thoroughly investigated for interferences with the thyroid hormone (TH) system. Most frequently, they either directly affect the thyroid gland or alter the hepatic TH catabolism. To capture these mechanisms rodent- and human-specific liver and thyroid models and their interconnection in the HUMIMIC Chip2 were established [1,2].

We show the reproducibility of these single-organ models, their optimization and intended future applications in the context of Next Generation Risk Assessment without the use of animal models.

Our newly characterized primary human hepatocyte-based liver spheroid model demonstrated increased formation of the glucuronidated TH catabolite (gT4) after a 6-day exposure to Rifampicin or beta-Naphthoflavone that was not seen in the previous model derived from HepaRG cells. The rat-specific gT4 formation was higher than in the human model and was induced by Pregnenolone-16-alpha-carbonitrile and beta-Naphthoflavone. Sulfotransferase-based TH catabolite formation remained specific to the human model.

To address the lacking T4 secretion by the human thyroid model, the concentration of thyroid-stimulating hormone was lowered from 0.1 to 0.001 mIU/ml, resulting in similar T4 and T3 secretion levels. TH secretion was reduced by the TPO-inhibitors Methimazole and Propylthiouracil, and the impact of, e.g., fluorine and iodine exposure as well as the NIS-inhibitor Sodium Perchlorate was investigated for the rat and human model.

Ongoing experiments evaluate chemicals in the coculture, focusing on the mechanism of thyroid toxicity caused by hepatic

metabolites and reduced thyroid toxic effects due to hepatic degradation of the active parent compound.

References

[1] Karwelat, D., Kühnlenz, J., Steger-Hartmann, T. et al. (2023). A rodent thyroid-liver chip to capture thyroid toxicity on organ function level. *ALTEX 40*, 83-102. doi:10.14573/altex.2108262

[2] Kühnlenz, J., Karwelat, D., Steger-Hartmann, T. et al. (2023). A microfluidic thyroid-liver platform to assess chemical safety in humans. *ALTEX 40*, 61-82. doi:10.14573/altex.2108261

Presentation: Poster

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Influence of diabetes on pancreatic ductal adenocarcinoma modelled in a pancreas-on-a-chip

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Introduction: In 2021, 537 million of the global adult population worldwide were living with diabetes [1]. Hyperactivity and abnormal proliferation of insulin-producing β-cells in response to peripheral insulin resistance can be intrinsically involved in cancer progression since insulin has been shown to directly stimulate pancreatic cancer cell growth [2]. Type 2 diabetes and obesity are associated with an elevated level of insulin and thus represent a common feature of low-grade inflammation in pancreatic ductal adenocarcinoma (PDAC). PDAC is a destructive disease with an unoptimistic prognosis, ranking 9th in the incidence of solid cancers and 4th for cancer-related deaths [3]. Three-dimensional cell cultures emerge as more relevant models towards organ-emulating constructs superseding the traditional two-dimensional format. Here, we created a perfusable microtissue-laden device to investigate the influence of diabetic microenvironment on PDAC.

Experimental procedure: In a 3D inkjet-printed acrylic device, a fugitive ink was extruded to subsequently serve as a U-shaped channel simulating the pancreatic duct. The sacrificial ink was then covered by a fibrin hydrogel or decellularized matrix, containing healthy or chemically-induced diabetic human pancreatic islets. The duct-mimicking channel was filled with human pancreatic epithelial cells either healthy or behaving preneoplastic abnormalities.

Results and discussion: We developed an amphicrine pancreas-on-a-chip model that recapitulate diabetic islet condition and allow immunostaining labelling in 3D, as confirmed by ELISA quantification of insulin secretion in response to glucose stimulation, and light-sheet microscopy. We will further analyze the link between the diabetic environment, cell activity (proliferation, migration) and the risk for development of pancreatic cancer.

Conclusion: Our approach allows to develop semi-automatized and standardized devices that can be used for multiple organ models. The advantages of hydrogel-based devices are the possibility of cell self-arrangement in the relatively large bulk of hydrogel, creating its own extracellular matrix, and maturation over time. The results are promising to generate glucose-responsive, functional Langerhans islet-based device for breakthrough research in the early stages of PDAC in diabetic patients.

References

[1] IDF Diabetes Atlas 2021, International Diabetes Federation (2021)

[2] Schneider, M. B. et al. (2001). *Gastroenterology 120*, 1263-1270.

[3] Jemal, A. et al. (2006). *CA Cancer J Clin 56*, 106-130.

Presentation: Poster

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MPS consisting of intestinal epithelial cells, macrophage, and bacteria for inflammatory bowel disease culture model

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Inflammatory bowel disease (IBD) is a chronic inflammatory disease caused by an over-activation of the intestinal immune system against intestinal bacteria, however IBD development mechanism is still vague. Because the rapid growth of bacteria induces cell-death, it is difficult to co-culture epithelial cells and bacteria on a dish. Here, we aimed to establish a tri-culture system of intestinal epithelial cells, immune cells, and bacteria developing a microfluidic device with a porous membrane sandwiched between two channels to create an IBD model. C2BBe1, a colon cancer-derived cell line, cells were cultured on a porous membrane in a microfluidic device. The device is equipped with electrodes to measure the electrical resistance between the channels across the membrane. Tight junction formation of C2BBe1 cells was confirmed by increase of trans-epithelial electrical resistance (TEER). On the other hand, RAW264, a mouse macrophage-like cell line, cells were cultured on the bottom of the lower channel. TEER of C2BBe1 could be maintained before addition of *E. coli* but was significantly decreased after the addition of *E. coli*

into the RAW264 channel, suggesting that the tight junction of C2BBe1 was impaired. It was also able to observe RAW264 cells phagocytosing *E. coli* on this device, suggesting that RAW264 is activated by *E. coli*. Expression levels of inflammatory cytokines such as *mTNF-α*, *mIL-1β*, and *mIL-6* were significantly increased in *E. coli*-activated RAW264 cells and those of *hIL-6* and *hIL-8* were increased in C2BBe1. Because several reports that TNF-α, IL-1, IL-6, and IL-8 were higher in the serum of the IBD patients correspond to our above results, our tri-culture system can be expected to be useful for investigation of the molecular mechanism and the drug screening.

Presentation: Oral

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The integrated development of blood brain barrier microphysiological system – from novel BBB MPS development to regulatory acceptance

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Microphysiological system (MPS), a new technology for *in vitro* testing platforms, have been acknowledged as a strong tool for drug development. In the central nervous system (CNS), the blood–brain barrier (BBB) limits the permeation of circulating substances from the blood vessels to the brain, thereby protecting the CNS from circulating xenobiotic compounds. At the same time, the BBB hinders drug development by introducing challenges at various stages, such as pharmacokinetics/pharmacodynamics (PK/PD), safety assessment, and efficacy assessment. To solve these problems, efforts are being made to develop a BBB MPS, particularly of a humanized type. We launched a BBB MPS project in the industry-government-academia MPS initiative of Japan (AMED MPS Project, 2017-; AMED Regulatory Science Project, 2022-). We first suggested minimal essential benchmark items to establish the BBB-likeness of a BBB MPS; these criteria support end users in determining the appropriate range of applications for a candidate BBB MPS [1]. We performed trial run using a two-dimensional (2D) humanized tricellular static transwell BBB MPS, the most conventional design of BBB MPS with human cell lines. We confirmed that 2D humanized tricellular static transwell BBB MPS is suitable for the functional assays of P-gp and BCRP. Currently we are attempting the practical application of 3D humanized BBB network MPS in collaboration with Osaka University, Japan [2]. This 3D humanized BBB network MPS attained transferrin receptor-mediated transcytosis, the highest needs from end users [3]. In this presentation, we will introduce the strategy of our BBB MPS project assembled from novel BBB MPS development to the regulatory acceptance.

References

[1] Kitamura, K. et al. (submitted).
[2] Figarol, A. et al. (2020). *Biomed Mater*. doi:10.1088/1748-605X/aba5f1
[3] Piantino, M. et al. (2022). *Mater Today Bio 15*, 100324.

Presentation: Oral

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On the way to a digital twin in preclinical studies – how automation and continuous data acquisition enable AI-based *in silico* models

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Performing *in vitro* assays using microphysiological systems requires a great deal of manual effort, which often hinders their use for industrial high-throughput testing. To overcome this, we have developed a robot for standardized long-term handling of TissUse's Multi-Organ-Chips. Our system allows the automation of assays featuring up to 24 chips with different degrees of complexity. The most complex chip currently automated is the HUMIMIC Chip4, which consists of a blood circuit and an excretory circuit and can sustain a long-term (several weeks) culture of up to four autologous organoids. Data are continuously recorded automatically during the execution. Examples are temperature data, pump and flow data as well as various optical readouts using brightfield and fluorescence microscopy. These include continuous morphological readouts such as number of cells, size changes or structural changes, but also chemical readouts such as pH values and value changes, CO_2 or oxygen concentration.

In this work, we will show that the advantage of automation goes beyond the mere reduction of manual work time, including

higher reproducibility and scalability, which is the basis for validation and standardization. The combination of holistic planning software, continuous data acquisition in databases and the integration of machine learning and artificial intelligence tools (GANs, CNNs) enables the implementation of virtual or so-called soft-(ware) sensors, which complement the directly measurable chip health metrics. By coupling this in-process data with hybrid AI models and expert knowledge, we are establishing the roadmap to a digital twin for preclinical studies.

Presentation: Oral

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Effect of adenine base editing on the function of a tissue-engineered vascular model of Hutchinson-Gilford progeria syndrome

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We tested the effect of adenine base editing (ABE) to correct the *de novo* c.1824 C > T point mutation in Exon 11 of the LMNA gene on the function of a tissue engineered blood vessel (TEBV) model of Hutchinson-Gilford Progeria Syndrome (HGPS). TEBVs were prepared with smooth muscle (SMCs) and endothelial cells (ECs) from patients with HGPS. These TEBVs exhibited loss of medial SMCs, calcification, reduced vasoactivity, extracellular matrix synthesis, and endothelial inflammation. Induced pluripotent stem cells (iPSCs) provided by the Progeria Research Foundation were transduced with lentivirus containing the ABE7.10max-VRQR adenine base editor and guide RNA. Editing HGPS iPSCs restored 97% wild-type allele at the HGPS mutation locus with minimal bystander editing and indel frequency. The edited iPSC-derived ECs and SMCs displayed 99% wild-type allele. Editing restored proliferation and reduced reactive oxygen species and DNA damage to levels found in healthy ECs and SMCs. Edited HGPS TEBVs displayed a full recovery of both vasoconstriction and vasodilation to levels not significantly different from healthy TEBVs. Base editing restored αSMA expression, MHC11 expression, and cell density in HGPS TEBVs to healthy levels. These results are consistent with published results showing improved SMC retention in base edited HGPS mice. Base editing also restored the reduced endothelial marker von Willebrand Factor (vWF) and cell cycle marker Ki67 expression displayed by HGPS TEBVs to healthy levels. Base edited HGPS TEBVs expressed reduced fibronectin and collagen IV expression that resembled healthy TEBVs.

To evaluate whether edited HGPS SMCs outcompete unedited HGPS SMCs, resulting in increased editing efficiency over time, TEBVs were prepared with various ratios of edited to unedited cells and structure and function measured after 21 days of perfusion. TEBVs prepared with a 50:50 and 75:25 edited: unedited ratio for ECs and SMCs showed improved vasodilation relative to HGPS TEBVs, whereas TEBVs made with 25:75 edited:unedited were unchanged relative to HGPS TEBVs. These functional changes were associated with increased SMC density and contractile protein levels. These results indicate that base editing of HGPS TEBVs recovers normal function and that editing of a fraction of the ECs and SMCs may improve vascular function.

Presentation: Poster

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3D printed organ-on-chip system for immunologic studies

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Alternative *in vitro* test methods are of growing interest because animal models often fail to predict outcomes of clinical trials. Organ-on-Chip (OoC) systems are being investigated and showed promising results demonstrating their great potential in minimizing animal tests [1]. First OoCs are commercially available but there is still a need for new fabrication approaches to customize microfluidic OoC devices.

In our lab an OoC system was fabricated from transparent polydimethylsiloxane (PDMS) using sacrificial 3D-printing with Pluronic F-127. However, cell adhesion, long fabrication time, and instability of the ink limited the outcome. Further studies established sugar-alcohol isomalt as a new suitable sacrificial material for 3D-printing-based fabrication of OoCs. Printing parameters for isomalt were validated and a first simple perfusion channel could be successfully produced via casting with PDMS in fast and reproducible way. Biocompatibility was confirmed by a selection of assays including adhesion of endothelial cells (ECs) within the cavities, rearrangement of EC morphology under shear stress and adherence of monocytes to ECs monolayers.

Based on these results an advanced membrane chip consisting of a second organ chamber, placed on top of the perfusion channel, separated by a porous membrane was produced. Applicability of this chip as OoC was shown in cell migration assays, where THP-1 monocytes migrated through the porous membrane (dependent on pore diameter) from the upper into the lower cavity in response to

a chemoattractant (N-formylmethionyl-leucyl-phenylalanine, fMLP). Furthermore, production of a conditioned medium from macrophages in response to lipopolysaccharide (LPS) was investigated as a model for immune response [2]. We could demonstrate two different cell lines being cultured in separated cavities, namely ECs in the lower and epithelial liver cells in the upper compartment.

The presented cost-effective and reproducible fabrication method for OoCs via 3D printing provides a straightforward approach towards novel alternative test systems for further investigations and developments. Demonstrating applicability of immunological assays further underlines the great potential of the devices shown.

References

[1] Tajeddin, A., and Mustafaoglu, N. (2021). Design and fabrication of organ-on-chips: Promises and challenges. *Micromachines (Basel) 12*, 1443.

[2] Chu, F. et al. (2013). *Cell Physiol Biochem 31*, 400-407. doi:10.1159/000343377

Presentation: Poster

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Well-defined extracellular matrices in organ-on-chips: A new approach

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Mechanical forces greatly impact various molecular-, cellular- and tissue-level biological processes, including morphogenesis, cell migration, proliferation, differentiation, and gene expression. Most prominent mechanobiological stimuli experienced by the cells *in vivo* involve shear stress caused by hemodynamic flow, compressive and tensile forces induced upon movement, and mechanical cues provided by the underlying extracellular matrix (ECM). Among these, the interactions between cells and their underlying ECM are particularly multifaceted as various ECM properties, including stiffness, composition, and topography have been shown to regulate the behavior and function of numerous cell types.

A considerable amount of knowledge on the interplay between cells and their ECM stems from traditional 2D cell cultures. These systems commonly utilize synthetic polymers of tunable mechanical, structural and biochemical properties coated on rigid glass and polystyrene substrates to mimic the *in vivo* ECM. While organ-on-chips (OoCs) present a strong alternative to traditional cell cultures by enabling the application of hemodynamic shear stress on cells and the controlled interaction of multiple tissue and organ systems, the current OoC technology still does not allow for the same level of ECM control as traditional culture systems. Currently, the ECM in OoCs is commonly established by coating the channel surfaces with an ECM protein solution, such as collagen or Matrigel. Such ECM integration approaches do not allow precise control and characterization of important on-chip ECM properties, such as its stiffness, surface topography, or ligand density.

In this work, we present a modular, foil-based, PDMS-free OoC that enables the creation of custom and defined ECM settings on chip. Due to its modular design, the system allows for the controlled functionalization of the porous cell culture membrane with polyacrylamide, a widely-known synthetic hydrogel of tunable mechanical, structural and biochemical properties. In contrast to the currently available systems, our OoC enables the independent control of the matrix stiffness, surface topography, ligand composition and density, which significantly broadens the range of possible biological investigations. Moreover, the functionality of the system is enhanced by the easy integration of electrodes, allowing for the real-time, non-invasive measurement of cellular properties.

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An improved differentiation protocol for human stem cell-derived islets

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Significant efforts are ongoing to develop stem cell-derived islets (SC-islets) for disease modeling, drug screening, and transplantation. However, it remains unclear to what extent SC-islets generated from current protocols recapitulate the physiology of human primary islets.

Here we present an optimized SC-islets differentiation protocol based on fine-tuning the WNT signaling pathway during the differentiation process. Our protocol led to SC-islets that showed a significantly increased population of Chromogranin A positive endocrine and C-peptide positive cells compared to benchmark protocols. The SC-islets can maintain glucose stimulated insulin secretion for 4 weeks *in vitro* with an average insulin secretion index (SI) of 1.45, and then the SI significantly decreases, reflecting a suitable time window for subsequent utilization. The SC-islets contain > 60% of insulin-producing cells, in which a small population of glucagon-insulin (> 15%) and somatostatin-insulin (> 10%) bi-hormonal cells exist. The bi-hormonal cells decrease during prolonged culturing *in vitro*. As expected, SC-islets increase insulin

secretion while decreasing glucagon secretion in response to an elevated glucose environment, reflecting their physiological potential in regulating glucose homeostasis similar to human primary islets. Furthermore, the oxygen consumption rate (OCR) during glucose stimulation increases in SC-islets, indicating they may have matured metabolic characteristics similar to primary islets. In alignment with the study in rodents, targeting the pyruvate kinase M2 in SC-islets *in vitro* improved their GSIS function without altering background insulin secretion. However, nutrient dependency studies showed that leucine and palmitate failed to magnify insulin secretion in SC-islets compared to human primary islets. Although our study shows progress in developing SC-islets, we also note the remaining pitfalls of SC-islets in mirroring primary islets.

In summary, we present an improved differentiation protocol for human SC-islets. These SC-islets can be a potential mini-organ source to fine-recapitulate the physiological function of primary human islets.

Presentation: Poster

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Human intestinal enteroids to identify antivirals targeting enteric viruses and the host immune response

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Human norovirus (HuNoV) is the main cause of viral gastroenteritis causing over 200,000 deaths every year, and currently there is no approved treatment or vaccine. Human intestinal enteroids (HIEs) have emerged as a unique opportunity to culture HuNoV. Before, the cultivation of enteric viruses such as human norovirus (HuNoV) was at large not possible, which hampered the development of therapeutics.

The aim of this research was to establish robust methods, using HIEs, to study HuNoV and aid in the search for therapeutics. First, HuNoV replication was evaluated in 2D monolayers or 3D cultures of differentiated HIEs from adult jejunum (J2) and fetal ileum (FI124) at 2-3 days post infection (dpi) by RT-qPCR. Replication of HuNoV GII.4 strains reached higher yields in the FI124 line and more reproducible infections in 3D, reaching a 2.5 $\log_{10}$ increase in HuNoV genomes. The reference compounds 2'-C-Methylcytidine (2CMC), 7DMA, favipiravir, CMX521, dasabuvir, nitazoxanide and rupintrivir were tested and showed anti-HuNoV GII.4 activity in 3D infected HIEs. Second, we infected differentiated HIEs seeded on transwell inserts with HuNoV GII.4, which yielded a 2 $\log_{10}$ increase in HuNoV genomes 2 dpi, 2CMC treatment was also successful. Transwell inserts provide access to both the apical and basolateral side of cultures, allowing the study of HuNoV shedding and thus indirectly transmission, for the first time. As immune cells closely interact with enteric viruses in the small intestine, we add human PBMC-derived macrophages apically and/or basolaterally. The infection and cell-to-cell viral spread is being studied by RT-qPCR and confocal imaging; cytokine secretion will be determined via ELISA. We can study *in vitro* the cellular toxicity, via an ATP-based luminescent cell-viability assay and confocal imaging.

We anticipate that organoid-based antiviral screens will take a central role in future drug development efforts. We are currently extending this model and setting up assays for human rotavirus, enterovirus A-71; the plan is to expand this list beyond the classic gastroenteric viruses. Additionally, we aim to study the interaction of viruses with the different intestinal/immune cell types via RNAseq and (live) imaging.

Presentation: Poster

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An innovative bioreactor platform: Fluidic shear stress reduces TNFα-mediated cartilage damage in a 3D model of degenerative joint disease

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Pathomechanisms of degenerative joint diseases such as osteoarthritis (OA) ultimately result in the breakdown of cartilage tissue. The precise underlying mechanisms of the origin and progression of OA remain unclear. However, mechanical forces are well-known as important modulators of joint health, with aberrant forces being primary etiologic determinants of cartilage degeneration.

Here, we analyzed the impact of mechanical forces, such as fluid shear stress (FSS), on cartilage formation and degradation in a standardized and defined manner.

In detail, we developed an *in vitro* 3D cartilage model using human bone marrow-derived mesenchymal stromal cells (MSC). We incubated the model in the OSPIN bioreactor platform to apply FSS-mediated mechanical stimulation via a perfusion cycle three times a day for 1.5 hours by circulating the culture medium with 10 dyn/cm² compared to 0 dyn/cm². Mimicking physiological mechanical stress during joint motion (10 dyn/cm²) reduced apoptosis and did not affect oxygen consumption but reduced glucose consumption and lactate production. In addition,

10 dyn/cm² enhanced chondrogenic gene expression, thereby decreasing MMP13 gene expression compared to 0 dyn/cm². Simulating the pathophysiology of OA, we stimulated the 3D cartilage model with the proinflammatory cytokine TNFα for 6 hours per day for 3 days under resting conditions and physiological mechanical stress applying FSS. Compared to TNFα treated cells under non-mechanical stress conditions, TNFα stimulation under perfused conditions did not affect cell survival but reduced apoptosis. Additionally, oxygen consumption was not affected. However, glucose consumption and lactate production as a measure of glycolysis were diminished. The expression of IL6 and soluble amounts of IL-6 were reduced, while soluble amounts of TNFα were enhanced. Furthermore, the expression of matrix-degrading enzymes was decreased at 10 dyn/cm² compared to 0 dyn/cm².

These data demonstrate that our bioreactor platform (i) enables the study of the effects of mechanical forces, and (ii) can serve as a preclinical tool for evaluating pharmaceuticals in a human pathophysiological setting.

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In vitro grafting of hepatic spheroids and organoids on a microfluidic vascular bed

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With recent progress in modeling liver organogenesis and regeneration, the lack of vasculature is becoming the bottleneck in progressing our ability to model human hepatic tissues *in vitro*. Here, we introduce a platform for routine grafting of liver and other tissues on an *in vitro* grown microvascular bed. The platform consists of 64 microfluidic chips patterned underneath a 384-well microtiter plate. Each chip allows the formation of a microvascular bed between two main lateral vessels by inducing angiogenesis. Chips consist of an open-top microfluidic chamber, which enables addition of a target tissue by manual or robotic pipetting. Upon grafting a liver microtissue, the microvascular bed undergoes anastomosis, resulting in a stable, perfusable vascular network. Interactions with vasculature were found in spheroids and organoids upon 7 days of co-culture with space of Disse-like architecture in between hepatocytes and endothelium. Veno-occlusive disease was induced by azathioprine exposure, leading to impeded perfusion of the vascularized spheroid. The platform holds the potential to replace animals with an *in vitro* alternative for routine grafting of spheroids, organoids, or (patient-derived) explants.

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Aptamer-modified nanopipettes for *in situ* monitoring of microphysiological systems

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Monitoring small molecules such as neurotransmitters is necessary to understand neuronal communication. Such measurements will lead to insights for better development of *in-vitro* models to study and potentially treat neurodegenerative diseases. Measurement of electrical activity inside of microphysiological systems has been reported [1]. However, a technological gap exists for selective, real-time measurements of neurotransmitters in low concentrations inside of 3-D models. To measure neurotransmitters such as dopamine or serotonin in picomolar to nanomolar concentrations, we have developed aptamer-modified nanopipettes. Aptamers are artificially designed DNA or RNA sequences that bind specifically to a target of interest. The analyte-specific aptamers are immobilized inside of a ca. 10 nm orifice of the nanopipette. Upon neurotransmitter diffusion inside of the nanopore, the aptamers capture the molecule, undergoing a characteristic conformational change, resulting in a measurable change in current [2].

We have demonstrated direct translation of these sensors into *ex-vivo* brain slices and have measured endogenous dopamine release upon electrical stimulation [3]. As the sensor is deployable in complex environments, we are working towards extending our sensing capabilities inside of microphysiological systems.

Control nanopipettes have been designed to ensure the specificity of the measured signal. These nanopipettes are functionalized with scrambled DNA sequences, which do not undergo structure switching in the presence of dopamine, while observing the same physiological milieu as the sensor. The placement in 3-D and standardizing the distance between the sensor and control nanopipettes is challenging, limited to a resolution of hundreds of micrometers, resulting in measurements of different local environments. We are working towards functionalizing double-barrel

nanopipettes, which would space the two nanopores at ~20 nm for optimal self-referencing.

Sensing neurotransmitters and other small molecules with high specificity at low concentrations inside of *in-vitro* systems would open a new axis of chemical analysis and help validate existing systems.

References

[1] Angotzi, G. N. et al. (2022). Integrated micro-devices for a lab-in-organoid technology platform: Current status and future perspectives. *Front Neurosci*.

[2] Nakatsuka, N. et al. (2021). Aptamer conformational change enables serotonin biosensing with nanopipettes. *Anal Chem*.

[3] Stuber, A. et al. (under review). Aptamer-modified nanopipettes for sensing endogenous dopamine release.

Presentation: Poster

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Establishment of an MPS model for routine testing of drug candidates and their effect on the intestinal barrier

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The potential for drug-induced gastrointestinal (GI) toxicity is significant, since the GI system is one of the first barriers which come in to contact with oral drugs. In pharmaceutical research, the complex behavior of human intestinal cells is traditionally investigated using 2D cultures, in which usually one cell type grows under static conditions. With the development of advanced microphysiological systems (MPS) more *in vivo* like conditions are generated which increase the predictive validity of these models.

Compared to a widely used Caco-2 cell 2D transwell model which mimics the intestinal barrier, the OrganoPlate 3-lane allows the recapitulation of the enterocyte cell layer of the intestinal barrier as the Caco-2 cells grow in a tubular structure through which the medium continuously flows.

The Caco-2 cells are known for their capability to build tight junctions. These connections are responsible for the maintenance of intestinal homeostasis and can be used as an endpoint, by measuring the Transepithelial electrical resistance (TEER), for the investigation of drug-induced toxicity.

The OrganoPlate was validated as a routine test system for the early prediction of drug-induced GI toxicity based on the measurement of the tightness of the cell layer by measuring changes in the TEER.

For this validation 24 well known compounds were selected. The TEER values were measured with the OrganoTEER 3-lane. 4 h and 24 h after treatment. In parallel the viability was determined after 24 h to be able to distinguish between a cytotoxic effect or a tight junction damage.

Overall, from the 24 tested compounds, 18 showed the expected outcome, i.e., the compound leads to either a decrease of the TEER of at least 50% with the highest tested concentration, for the positive control compounds, or the TEER value remained stable after treatment with non-GI-toxic compounds. In summary this Organ on a chip system allowed the recapitulation of the human intestinal GI barrier and enabled a fast and robust assessment of drug-induced damage in the GI tract.

Presentation: Poster

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Tubule-on-a-chip: Culture and analysis of a novel immortalised human distal convoluted tubule cell line in an organ-on-a-chip system

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Introduction: The kidney maintains blood pressure and electrolyte balance through the entwined actions of the tubular nephron segments. Recent investigations into rare monogenic diseases, specifically Gordon and Gitelman syndromes, occurring in the distal convoluted tubule (DCT) segment of the kidney, underline the critical physiological role of this segment in regulating blood pressure. Despite its crucial significance, no well-characterized and independently validated human DCT cell lines have been identified, with only a limited number of murine DCT cell lines available for *in vitro* studies. To date, none have been incorporated into the Organ-on-a-Chip (OOaC) system. This study aims to employ a novel, immortalized human DCT cell line into a multi-channel OOaC system, with the ultimate goal of creating the first human DCT Tubule-on-a-chip (TOaC).

Method: Immortalised hDCT cells characterised in our lab (gifted from Dr Kusaba, Kyoto Prefectural University of Medicine, Japan) were cultured as previously described (Ikeda et al., 2020). Cells were applied in a three-lane, micro-plate-based microfluidic chip platform OrganoPlate (Mimetas, Leiden, The Netherlands) following manufacture's protocol with modifications on the constitution of the extracellular matrix gel. Tubules formed in the OrganoPlate channel on an average of 5-7 days of culture. TOaCs were fixed using 4% w/v formaldehyde-PBS for 15 min at room temperature or lysed with TRI reagent for RNA isolation. Segment specific marker expression was confirmed by staining with fluorescently tagged antibodies/lectins and qPCR.

Result: Barrier integrity assay in the OrganoPlate using fluorescent probes showed tight junctions between cells. qPCR of the cell lysate detected these TOaCs expressed the DCT-specific marker NCC (*SLC12A3*). NCC antibody confirmed positive staining localized to the apical membrane.

Discussion: These preliminary data demonstrate that this novel hDCT cell line is able to form tubule-like structures in the OrganoPlate. Given this cell line were derived from primary human DCT cells, we propose this TOaC model will better reflect *in vivo* human DCT physiology compared to iPSC-based systems. Tubular function will be validated by ion transport assays and pharmacological responses. In the future, we aim to create patient-specific TOaC from urine-derived cells and conduct therapeutic optimisation, thereby bringing true personalised medicine to nephrology.

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Clostridiodes difficile infection in a primary human intestinal gut-on-chip

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Introduction: Clostridiodes difficile (*C. diff.*) is one of the major pathogens of gastrointestinal tract infections and could cause life-threatening symptoms in patients [1]. Pseudomembranous colitis is associated with loss of barrier integrity and inflammation and caused by toxins secreted by *C. diff.* as a result of an intestinal overgrowth of these bacteria subsequent to antibiotic treatment [1]. There is still a lack of respective preclinical disease models which sufficiently reflect the human *in vivo*-situation [2]. Microphysiological gut-on-chip systems of patient-specific primary cells will provide more detailed insights into infection processes, immunological reactions and potential preventive or therapeutic measures.

Project Outline: Human intestinal organoids derived from adult stem cells isolated from the duodenum and colon descendens of healthy and diseased patients were used to generate gut-on-chip models. The intestinal model is used for studying the infection process of *C. diff. in vitro*. To increase model complexity, endothelial cells, macrophages as well as neutrophils will be included, thereby, developing an immunocompetent stem cell-based gut-on-chip model. In the gut-on-chip we will establish conditions of hypoxia to follow colonization with living obligate anaerobic *C. diff. in vitro*. Eventually, neutralizing antibodies will be tested for their ability to prevent colitis phenotypes induced by *C. diff.*

Preliminary results: Stem cell-derived human intestinal cells were cultured in microfluidically perfused biochips and treated with *C. diff.* toxins A (TcdA) and B (TcdB), which both induced toxin-specific disruption of the epithelial cell layers. The aim of this study is to develop a microphysiological gut-on-chip model which can be used as a tool to study *C. diff.* infection and to investigate possible preventive or therapeutic options based on toxin-neutralizing antibodies.

This work has received support from the IMI2/EU/EFPIA Joint Undertaking Inno4Vac grant n° 101007799.

References

[1] Abt, M. C., McKenney, P. T. and Pamer, E. G. (2016). Clostridium difficile colitis: pathogenesis and host defence. *Nat Rev Microbiol 14*, 609-620.

[2] Ewin, D., Birch, W. D. and Moura, I. B. (2023). In vitro models to study Clostridioides difficile infection: Current systems and future advances. *Curr Opin Gastroenterol 39*, 23-30.

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A PK/PD translational microphysiological system to explore anti-cancer therapies efficacy on 3D tumour spheroids and patient derived organoids

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Developing effective oncology therapies involves defining the right schedules to minimize side effects and maximise efficacy. This requires an accurate understanding of the pharmacokinetic/pharmacodynamic (PK/PD) relationship of the compound(s) [1]. *In vitro* experiments are mainly performed at fixed concentrations and do not explore PK/PD relationships, which represents a limit to their translational relevance [2].

To explore human tissue-specific responses we have developed a microphysiological system (MPS) able to mimic human PK/PD efficacy relationships following mono or combination therapy on 3D tumour spheroid models and patient derived organoids.

We first recapitulated the *in vivo* PK/PD relationship for a PI3K inhibitor (BYL719- t1/2 = 6 hours, equivalent to 50 mg/kg murine oral dose), used to treat an A549 non-small cell lung carcinoma model. Following BYL719 exposure in the MPS platform, cellular p-AKT levels (biomarker of PI3K pathway activity) fell by up to 50%, and then recovered to pre-dosed levels over a 24-hour period, for 3 days in a row. This effect is not captured in standard *in vitro* bolus experiments.

Next, we explored whether the MPS could recapitulate *in vivo* effects of combining topoisomerase inhibitors with DNA-damage-response inhibitors (DDRi) in the treatment of 3D colorectal tumour models – spheroids and multiple donor patient-derived organoids. We mimicked the murine PK profiles for 6 days with the delivery of mono and combination therapies (SN38 Cmax = 20 nM, DDRi Cmax = 162 nM. Viability assays and fluorescent labelled biomarker expression evaluated by confocal microscopy demonstrated the benefits of combination therapy, with efficacy rates consistent with *in vivo* observations, in contrast to static 2D *in vitro* cultures.

Our MPS platform is able to mimic animal and human PK profiles thus potentially avoiding many failures of novel therapies which are due to a missing physiologically relevant link between preclinical and clinical data. By tailoring individualized PK profiles and therapeutic schedules, we introduce a valuable tool and strategy in the pursuit of personalized medicine for the treatment of cancer.

References

[1] Malik et al. (2021). doi:10.3389/fcell.2021.721338
[2] Sonhheimer-Phelps et al. (2019). doi:10.1038/s41568-018-0104-6

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Validation of primary human hepatocyte spheroids for early ADME assessments

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During the drug development process, it is required to evaluate both toxicological and ADME properties of all new drugs. This includes the study of drug-drug interactions which may alter the toxic potential or metabolism of the drug. In order to investigate if metabolites are affected via drug-drug interactions, highly selective probe substances, inhibitors and inducers are needed. However, current inhibitors for Cytochrome P450 (CYP) enzymes are not entirely selective or not functional for long-term inhibition, precluding the analysis of the turnover of slowly metabolized drugs.

Here we show that siRNA-dependent knockdown of selected metabolizing phase I enzymes in spheroid cultures of primary human hepatocytes (PHH) is highly selective, thus representing a novel and promising way of studying drug-drug interactions. We have shown successful depletion of several CYP isoforms using Luminex gene expression analysis. Our analysis further revealed that the knockdowns had no significant effect on the gene expression of other phase I and II drug metabolizing enzymes, as well as drug transporters. Therefore, our data indicate a high specificity of the siRNA-mediated approach. CYP specific metabolite formation from probe substances using PHH spheroids correlated with the gene expression analysis, showing less metabolism after successful knockdown of the respective drug metabolizing enzyme with little to no effect on the metabolic activity of other enzymes.

We anticipate our analysis being the starting point for the study of drug-drug interactions in a more reliable and phenotypic relevant system. By using spheroid cultures of primary human hepatocytes, we hope to provide a basis for more human relevant drug development studies that overcome the limitations of current models. In conclusion, our data will help to refine the evaluation of drug safety, thus improving the cost-intensive and unreliable process of drug development.

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Development of a novel micro-engineered heart tissue platform on chip with multicellular biomimicry

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Cardiovascular diseases (CVD) are the leading cause of death globally and there is currently no cure available. The efficient generation of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) enabled their use for disease modeling, drug discovery and regenerative medicine in CVD. Moreover, in order to enhance their maturity *in vitro*, several three dimensional (3D) *in vitro* systems, such as Engineered Heart Tissues (EHTs) have been developed and proven to be efficient in improving CM maturation [1,2]. Nevertheless, conventional EHT models require high cell numbers, which makes them expensive, and lack flow, a key element in heart function. Additionally, recapitulation of the complex CM and non-CM interactions as they occur *in vivo* is key to further enhance CM maturation [3]. Heart-on-chip (HoC) systems allow for precise control of flow and facilitate dynamic adjustments of the required microenvironment. Nevertheless, current microfluidic HoC do not feature flexible pillar-based EHTs that allow for precise assessment of contractile forces and do not exhibit the self-organization of the cardiac tissue as occurs *in vivo*. Moreover, their arduous use, complex fabrication and limited robustness hamper their use. Therefore, in this study we designed a novel and user-friendly HoC platform featuring four micro-EHTs (µEHTs) consisting of 50,000 cells each. We show that the addition of flow improves contractile performance of the µEHTs. Moreover, we demonstrate for the first time that culture of cardiomyocytes (CMs), endothelial cells (ECs), smooth muscle cells (SMCs) and fibroblasts (FBs) results in self-assembled µEHTs, where ECs form a wrapping layer around the tissue, better mimicking the *in vivo*-like CM-EC interface while preserving direct cell-cell contact. We found that this multicellularity enhances cardiac performance by improving contractile force, post-rest potentiation and maintenance of high frequency pacing and also delays drug response compared to µEHTs with only CM:FBs. We

believe that this innovative HoC model facilitates accurate disease modeling and cardiac toxicity screening.

References
[1] Hansen, A. et al. (2010). *Circ Res 107*, 1.
[2] Ribeiro, M. et al. (2022). *J Pers Med*.
[3] Giacomelli, E. et al. (2020). *Cell Stem Cell*.

Presentation: Oral

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Combining tumor-on-chip technology and metabolic imaging to monitor treatment efficacy of cancer therapies on patient derived microtumors

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Tumor-immune interactions play a crucial role in tumor prognosis and therapy resistance. In the area of personalized medicine, patient-derived tumor tissues represent a promising tool to access individual drug and dose responsiveness. However, the role of immune evasion and the tumor microenvironment are still poorly understood. Using an organ-on-chip model allows the investigation of interactions between tumor (or organoids) with components of the immune system. Conventional readouts such as gene or protein expression assays, often lack in their capacity to fully capture dynamic conditions. These conventional assays often require the application of additional reagents, destructive sample processing, do not allow for spatial resolution or only represent endpoint readouts. Using non-invasive imaging techniques such as Fluorescence Lifetime Imaging Microscopy (FLIM) allowing for marker-free *in situ* monitoring. FLIM is non-destructive and does not require any additional factors and is especially sensitive to metabolic changes by targeting the endogenous fluorophores NADH and FAD.

Our aim is to establish real-time observations of Patient Derived Microtumors (PDMs) in a microfluidic platform to assess their responsiveness to different cancer therapies. We initially established a protocol using MCF-7 spheroids to monitor the metabolic response in live 3D cell aggregates by FLIM. The FLIM data was compared to a conventional cell death assay (LDH) to evaluate FLIM sensitivity and earlier detection of metabolic changes than using conventional assays. Furthermore, the protocols were translated to include PDMs in a tumor-on-chip 3D microenvironment which was monitored over time. Metabolic changes targeting the ratio between glycolysis or oxidative phosphorylation were identified.

In the future, FLIM will be further implemented to monitor patient-specific treatment efficacies to enable the monitoring of tailored cancer therapy approaches. Advancing the complexity of the tumor-on-chip platform might reveal more information about the contribution of the tumor-immune interaction.

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Functional assessment of hiPSC derived brain organoids to study the effects of chemical exposure and electrical stimuli on synaptic plasticity

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Cognitive endpoints are used in human and animal studies to determine if a chemical will cause neurodevelopmental toxicity in drug screening. Currently, there are no *in vitro* assays that can be used to assess cognition, therefore, to determine the effect a chemical has on learning and memory, animal models are used. Recent advances in cell culture including Microphysiological Systems (MPS) allow more physiologically relevant modeling of the cellular processes *in vitro* and bring the *in vitro* models closer to *in vivo*. Our lab established a highly standardized and reproducible hiPSC derived brain organoid MPS consisting of all neural cell types found in the central nervous system, myelinated axons, and spontaneous local field potentials [1]. Using this MPS we aim to assess the extent of learning and memory in a dish to determine the effect a chemical has on synaptic plasticity so this platform can be used for high throughput drug screening. To research this, we have been differentiating brain organoids for up to 12 weeks and characterizing spontaneous and modulated electrical activity using live calcium imaging and HD MEAs. In addition, we characterized the molecular components of early synaptogenesis, including immediate early genes (IEGs) and miRNAs over time in conjunction with chemical exposure by immunohistochemistry and RT-PCR. Organoids showed spontaneous electrical activity using both HD MEAs and calcium imaging and overtime showed evidence of highly interconnected network formation within and between organoids. Additionally, we saw time-point specific expression of

IEGs over development, which are known to be involved in synaptic plasticity. Additionally, our results show that receptor agonists and antagonists impact IEG expression and electrical activity. In the future, we plan to build upon this model to study how long-term potentiation is affected by chemical and electrical exposures to develop a model to perform high throughput chemical screening to assess functional changes in synaptic plasticity. An *in vitro* cognition assay developed for hiPSC brain organoid MPS can fill the gap between human and animal data while increasing throughput for drug screening to test for the effect of chemicals on neurodevelopment and neurodegeneration.

Reference

[1] Pamies, D. (2017). *ALTEX 34*, 362-376. doi:10.14573/altex.1609122

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Beating heart-on-a-chip: Integration of electrodes to measure contractility of cardiac spheroids

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Human cardiac spheroids hold great potential to study cardiac therapies *in vitro*. We are developing multi-organ microphysiological system (MPS) for cardiometabolic research using cardiac spheroids and liver and pancreas organ models [1,2]. However, there are no commercially available technologies to evaluate contractility, a key heart function, of 3D spheroids cultured on chip. Indeed, the lack of disease relevant readouts critically limits the use of cardiac spheroids to study efficacy of cardiac therapies. Therefore, we have embarked upon integrating electrodes on chip to follow effects of drugs on cardiac contractility.

In this study, we embedded custom-designed multi-electrode arrays on the HUMIMIC Chip2 platform. The sensing electrodes are made of PEDOT:PSS, an electrically conductive and biocompatible polymer. This polymer is characterized by low thermal noise and superior charge injection when compared to standard metal electrodes and can be easily ink-jet printed on plastic at high-throughput scale. Moreover, its optical transparency (typically > 85%), allows simultaneous monitoring of the culture by using standard microscopy techniques making the PEDOT:PSS an ideal material for the intended application.

First, we confirmed the biocompatibility of the electrode-integrated chip. 3D cardiac spheroids formed of human induced pluripotent stem cell (hiPSC) derived cardiomyocytes and human primary fibroblasts showed similar viability on electrode-chips as in the standard well plate culture. Next, we cultured hiPSC-derived cardiomyocytes on the electrode-chip as a monolayer and monitored their electrical activity for 14 days. The recordings were implemented by simultaneously measuring electrical activity from 8 single-ended sensing sites, with a sampling rate of 250 samples/second. Upon controlled administration of isoprenaline, a synthetic catecholamine that increases the heart rate and cardiac contractility the spontaneously contracting cardiomyocytes increased their beat rate. This positive chronotropic effect did not only prove the expected *in vivo* like response [3] but demonstrated functionality of the electrodes. Development of disease-relevant readouts, such as electrode-chip technology, will utilize the full potential of the MPS and shall aid the discovery of new therapies.

References

[1] Bauer, S. et al. (2017). *Sci Rep 7*, 14620.
[2] Casas, B. et al. (2022). *PLoS Computat Biol 18*, e1010587.
[3] Weiss et al. (2013). *Circ Res 113*.

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DSS-induced colitis-on-chip model to study the therapeutic potential of the secondary bile acid lithocholic acid *in vitro*

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Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a major health threat to humans and is associated with intestinal permeability, inflammation and microbial dysbiosis. Treatment of mice with dextran sodium sulfate (DSS) to induce a colitis phenotype is an established model to study IBD [1]. However, the validity of animal models for humans is limited due to interspecies variability, resulting in challenging extrapolation to the human situation. Secondary bile acids (sBAs) have been discussed as a potential treatment option for IBD. In homeostatic conditions, the sBA lithocholic acid (LCA) promotes intestinal barrier integrity, anti-inflammatory effects, and reduced apoptosis in intestinal cells [2]. *In vivo*, the levels of LCA were drastically reduced during IBD, leading to the speculation of a causative link between IBD and reduced sBA levels. We leveraged a recently established human intes-

tinal MPS [3] to reproduce IBD-like conditions by DSS treatment and to investigate the therapeutic potential of LCA to ameliorate the disease-like phenotype *in vitro*.

The intestinal model was assembled as previously described [3] in the Dynamic42 biochip. Human umbilical cord vein endothelial cells and macrophages were seeded in the top chamber and Caco-2 cells in the bottom chamber. Both channels were perfused to induce villus-like structures in Caco-2 cells. Intestinal cells were treated with DSS and LPS for up to 48 h. Formation of tight junctions (E-cadherin, ZO-1, VE-cadherin), intestinal permeability, villus height and cytokine levels were investigated for their modulation by LCA.

In the DSS-induced colitis model we observed a reduced expression of tight junction proteins, increased permeability, compromised villus formation and elevated cytokine release. Treatment with LCA rescued the majority of these effects in the IBD model and resulted in the establishment of an anti-inflammatory milieu.

Our findings underline the potential of LCA to interfere with IBD conditions. In future studies, we will explore the mechanistic details of sBA-mediated IBD phenotype amelioration in a human stem cell-based model reflecting patient-specific IBD genotypes.

References

[1] Okayasu, I. et al. (1990). *Gastroenterology 98*, 694-702.
[2] Sheng, W. et al. (2022). *Front Pharmacol 13*, 910493.
[3] Maurer, M. et al. (2019). *Biomaterials 220*, 119396.

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Cancer-mediated chemoattraction drives axonal guidance and excitability of 3D sensory neurons in a compartmentalized innervation chip

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Potential links between the Tumor Microenvironment (TME) and pathological phenotypes of neurons from the peripheral nervous system (PNS) have been elucidated previously, *in vivo* [1]. Particularly, when cancers metastasize to, e.g., the bone, close proximity sensory innervation is driven towards pathological sprouting, which has been posed as a rationale for increases in pain experienced by cancer-patients. We set out to recapitulate this phenomenon in compartmentalized glass 3D culture chips (INV-Chip) that enable investigation of specific aspects of cancer-neuron interactions, namely: paracrine factor release guiding neuronal outgrowth, alterations in morphology upon establishing contact (ectopic sprouting) and changes in sensitivity towards depolarizing stimuli (neuronal excitability). The developed INV-Chip serves as a multimodal platform for the manipulation and complementary analysis of solely axons (separated from their cell bodies by microtunnels [2]) innervating tumor-like structures in 3D. Utilizing a selectively laser etched (SLE) glass chip bonded on top of a customized high density microelectrode array (MEA) allows imaging and simultaneous measurement of excitability along nerve fibers, non-invasively, under a range of physiologically relevant conditions. After benchmarking with neurotrophic factor NGF – we successfully demonstrate that neurons also consistently cross the micro tunneled barrier through chemoattraction mediated by axonal guidance ques present in the TME of cancer spheroids cultured in the distal compartment. Additionally, crossing sensory fibers could be chemically excited by distal application of known pain-inducing agonists (capsaicin and bradykinin) only when co-cultured with cancer cells. This is, to our knowledge, the first system that showcases morphological and electrophysiological analysis of 3D-innervated tumor tissue *in vitro* and may open the door to a plethora of studies into understanding the effects of complex neuro-oncological crosstalk on PNS electrophysiology. Furthermore, the developed platforms do not only serve neuro-oncological research but have the potential to satisfy the needs of any researcher trying to recapitulate the complexity of innervated tissues *in vitro*.

References

[1] Jimenez-Andrade, J. M. et al. (2010). Pathological sprouting of adult nociceptors in chronic prostate cancer-induced bone pain. *J Neurosci 30*.
[2] Vysokov, N. et al. (2019). The role of NaV channels in synaptic transmission after axotomy in a microfluidic culture platform. *Sci Rep 9*.

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Single-cell resolution spatial transcriptomics on high density CMOS MEA chips

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Microelectrodes are great tools for investigating electrophysiology in MPS, such as Alzheimer's disease (AD) or Parkinson's disease (PD) models. However, understanding the transcriptome of these polygenic diseases are equally important. Current methods for *in vitro* spatial transcriptomics (like FISH) on neural culture are incompatible with performing live-cell spatial electrophysiology, as they require neuron tissues to be fixed and permeabilized for barcode introduction. Therefore, to better understand the mechanism

of AD and PD, and its connection to sporadic or inherited genetic loci, we need a platform that can capture single, spatial electrophysiology and transcriptomics data at the same time.

We have developed High Density Multielectrode Arrays (HDMEA) that can take simultaneous recordings of neural cultures with single-cell spatial resolution (electrode pitch of 15 µm) and temporal resolution of 30 kHz. All > 16k electrodes on the chip can be addressed, and 1024 electrodes can be used simultaneously. This chip is used in neural network monitoring, where we previously used the chip's integrated amplifiers to record *in situ* from cultured hippocampal neurons for more than an hour, showcasing its capability for long-term electrophysiology monitoring [1].

Here, we present our HDMEA chip as an *in vitro* spatial cellular transcriptomics platform. We performed spatially targeted single cell electroporation on Human Dermal Fibroblasts using the chip's integrated stimulator to introduce spatially tagged oligonucleotide barcodes with multiplexing. We report the successful introduction of the fluorophore-tagged ssDNA barcodes into single cells on pre-defined locations on the chip, with 78% of cells successfully delivered with barcodes. We are currently performing *de novo* scRNA-seq on these location-tagged cells to confirm the unique introduction of spatial barcodes.

Combining both sources of information, this chip will enable a wide range of investigations that are beyond the current capabilities of conventional methods: network-wide electrophysiology studies between connected diseased and healthy neurons, screening drug targets for AD or PD neurons, investigating transcriptomic effects of electrical stimulation on disease cells, or pathological studies for functional impact of disease-altered neural circuits.

Supported by FWO PhD Fellowship Strategic Basic Research (1S70723N)

Reference

[1] Miccoli, B. et al. (2019). *Front Neurosci 13*. doi:10.3389/fnins.2019.00641

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Are glycol ethers neurotoxic for humans? An *in vitro* and *in silico* evaluation

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Organic solvents are commonly found in industrial and consumer products, and exposure can lead to cognitive impairment. More than 10% of workers are estimated to have occupational exposure to at least one solvent [1], which is in addition to exposures at home. Recent research suggests that organic solvents interact with lipophilic areas of protein receptors and cause lipid peroxidation leading to failure in energy production [2,3]. Whereas the neurotoxicity of organic solvents such as toluene, trichloroethylene and n-hexane has been recognized, the neurotoxicity of most solvents currently on the market have not been evaluated. The OECD guidelines, based on animal experiments, only require specific neurotoxicity testing if a trigger is found in single-dose or repeated-dose toxicity studies. However, recent technological developments have led to human cell-based testing methods, such as spheroid cultures, organoid cultures and on-a-chip technologies (also called microphysiological systems (MPS)), that better represent human physiology [4] and fill the gaps in neurotoxicity assessment. The neurotoxicity of 3 glycol ethers (EGME, PGBE, PGME) and their respective metabolites (MAA, 2BPA, 2MPA) was evaluated using our induced pluripotent stem cells (iPSCs)-derived 3D human brain MPS, also called BrainSpheres, which contains neurons, astrocytes, and oligodendrocytes. BrainSpheres were repeatedly exposed to the solvents for 7 days. Cytotoxicity was evaluated by the MTT assay in BrainSpheres exposed to concentrations ranging from 1 to 150 mM of solvents. PGBE was the most cytotoxic compound tested. Gene expression and immunostainings for cell type-specific markers, myelin and synaptic markers suggest that neurotoxicity is triggered by these solvents at concentrations fitting with predicted *in silico* brain concentrations indicating that neurotoxicity could be produced in humans. Mechanistic information on organic solvent neurotoxicity will be extracted from planned proteomics and metabolomics analyses, which will be used to identify potential key events for the construction of a new Adverse Outcome Pathway (AOP) relevant for the nervous system.

References

[1] Arnaudo, B. et al. (2013). http://www.inrs.fr/dms/inrs/CataloguePapier/DMT/TI-TF-207/tf207.pdf
[2] Revilla, A. S. et al. (2007). doi:10.1016/j.tiv.2007.01.012
[3] Fukumori, R. et al. (2013). doi:10.1371/journal.pone.0069718

[4] Bal-Price, A. and Meek, M. E. (2017). doi:10.1016/j.pharmthera.2017.05.006

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Evaluation of two complex 3D *in vitro* human alveolar co-cultures for prediction of lung inflammation and toxicity

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Inhalation is an important delivery route for candidate drugs to treat respiratory diseases such as asthma and chronic obstructive pulmonary disease. The inhaled route for delivery has the benefit of achieving direct access to the lung but is often impacted by clinical dose level limitations due to lung histopathological findings or functional effects in safety *in vivo* studies. Our aim is to predict and mitigate lung safety concerns early in the drug discovery process to ensure the progression of compounds with the right safety profile. Of key importance is the ability to predict lung inflammatory responses. To do this sensitive and predictive lung *in vitro* models which include immune components are important. To this end we have evaluated two *in vitro* human alveolar co-cultures, MatTek EpiAlveolar™ 3D-model and AlveoliX human ^{AX}Lung-on-chip model, for prediction of lung inflammation and toxicity. The EpiAlveolar™ 3D-model is a static culture composed of primary alveolar, endothelial, fibroblasts and THP1 derived macrophage cells, whilst the AlveoliX chip includes alveolar epithelial, endothelial and PBMC cells (Sengupta, 2022). The ^{AX}Lung-on-chip also enables application of physiological stretch to mimic breathing. Endpoints utilized for evaluation were barrier integrity (TEER), cytokine secretion and viability measurements. In the EpiAlveolar™ 3D-model we have tested a set of compounds with various modalities including known *in vivo* non-toxic and toxic substances to evaluate the predictivity for *in vivo* inflammatory histopathological findings. In collaboration with AlveoliX AG a subset of these substances has been tested and the cytokine and barrier profiles were evaluated. The results reveal that both models are able to distinguish between toxic and non-toxic compounds, however with differences in terms of response window and data variability in the utilized study designs that will be presented. The ^{AX}Lung-on-chip model had significantly more sensitive cytokine response indicating a superior ability to detect inflammatory processes whilst the MatTek 3D-model had greater sensitivity in its TEER response indicating its suitability to investigate potential lung barrier toxicants. This study indicates a place for both models in an inhaled safety strategy cascade for novel therapeutics with the potential to prioritize model system depending on the safety question under investigation.

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Tumor-on-chip model to decipher the effect of nanoparticle-mediated photothermia (NP-PTT) on tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC)

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Pancreatic Ductal AdenoCarcinoma (PDAC) represents > 90% of pancreatic cancer cases and is characterized by very poor prognosis partly due to high therapy resistance [1]. Mostly, this resistance is attributed to the extensive fibrotic stroma with enhanced desmoplastic effect within tumor microenvironment that impedes drug delivery [2]. Thus, there is an urgent need to develop both effective treatments to overcome this barrier & relevant *in vitro* models to test novel strategies. *Nanoparticle-mediated photothermal therapy* (NP-PTT) presents a promising technique for adjuvant cancer treatment, through local heating of malignant cells, matrix denaturation, and T cell recruitment among others [3]. Herein, we implement for the first time NP-PTT in PDAC tumor-on-chip (ToC) to both closely model the PDAC *in vivo* microenvironment and understand the effect of NP-mediated therapies and their potential as synergized therapies (with chemo-/immunotherapies).

We model our ToC with human PDAC cells (PANC-1) cultured in collagen type I matrix. To evaluate the NP-PTT effect on both matrix & cancer cells, we study matrix remodelling and cell fate as a function of temperature and post NP-PTT resting times.

First, we developed the PDAC-on-chip model with formed tumor spheroids (~100 µm) from single cancer cells in collagen, exhibiting invasive phenotype. This indicates progressive tumor formation with matrix invasion, closely mimicking *in vivo* conditions. Successful on-chip NPs diffusion into the matrix, variable NP concentration and laser power allowed for fine PTT tempera-

ture control: 37-55°C. Subsequent exposure to NP-PTT invoked increasing tumor cell death (up to 80%) as well as matrix degradation following increasing temperature (up to 48°C). The effect of post-treatment rest time is currently under investigation.

Successfully implemented NP-PTT in PDAC-on-chip showed that NP-mediated heating allows to finely control ToC system, promoting temperature-dependent cell death & matrix degradation. Our next steps consist in elaborating the ToC with stellate cells to induce crucial *in vivo* desmoplastic reaction, along with further investigating matrix remodelling, heating-induced EMT profile & potential T cell recruitment.

References

[1] Cai, J. et al. (2021). Advances in the epidemiology of pancreatic cancer: Trends, risk factors, screening, and prognosis. *Cancer Lett*.

[2] Yang, H. et al. (2021). Photosensitizer nanoparticles boost photodynamic therapy for pancreatic cancer treatment. *Nanomicro Lett*.

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On chip pancreatic cancer modelling with decellularized extracellular matrix from pancreas

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With a survival rate of only 9% five years after diagnosis, pancreatic ductal adenocarcinoma cancer (PDAC) has one of the worst prognoses today [1]. This low rate is due to the absence of specific symptoms and sensitive biomarkers, leading to a late diagnosis, and the low efficacy of current chemotherapies. Development of new, appropriate disease models are urgently needed to discover new biomarkers and identify new possible treatments. To date, most models focus only on cellular aspect and forget the microenvironment, which plays an important function in pancreatic cancer. Here we propose to use hydrogels obtained from porcine decellularized extracellular matrix as a way to model the pancreatic microenvironment.

Porcine pancreases were successfully decellularized and resuspended as a hydrogel to generate pancreatic decellularized extracellular matrix (pdECM). Immunostaining, mass spectrometry-based proteomic and cytokine arrays were used to determine its composition and proved the presence of structural and secreted proteins. pdECM hydrogel was used to develop a model for PDAC. First, healthy pancreatic exocrine cells cultured on top of pdECM developed as organoids; tumoral cells cultured in the same manner formed tumoroids. Second, endothelial cells and pancreatic stellate cells were culture inside the hydrogel and formed an endothelial network. Finally, the biological system was adapted into a microfluidic chip previously developed in our group [2]. This microfluidic chip forms a circuit in which there is a restriction, allowing trapping of a single 3D structure. pdECM containing endothelial cells and a tumoroid was perfused on chip allowing trapping of the tumoroid inside the restriction. The tumoroid was surrounded by the microenvironment and the chip was under flux.

In conclusion, pancreatic dECM offers a versatile and physiological scaffold for pancreatic cell culture, and development of a complete PDAC model with stromal component and ECM. Integrating the matrix inside a serpentine chip allows individual observation of tumoroids within its microenvironment and could be used as a drug-screening platform for PDAC.

References

[1] Sung, H. et al. (2021). *CA Cancer J Clin*.

[2] Quintard, H. et al. (2021). *BioRxiv*.

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Combining microtunnel devices and actuator chips: Instructive microenvironments for neural networks

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Brain-on-Chip (BoC) devices are promising microfluidic models for furthering our knowledge of neurodegenerative diseases and potential therapeutic interventions. Of particular interest is the use of these devices for studying the effects of mechanical and geometric cues on neural cultures. In line with this, we have previously demonstrated that so-called actuator chips can be employed to exert nanoscale mechanical loads on cortical networks derived from rat primary cells by microfluidic pneumatic deformation of a polydimethylsiloxane (PDMS) membrane [1,2]. Furthermore, we have demonstrated that microscale tunnels can be used to guide axonal growths of SH-SY5Y neuroblastoma cells [3]. In this study, we combine the mechanical cues from actuator chips with the geo-

metric cues provided by microtunnels to develop one instructive microenvironment to control the development of neural networks derived from Ngn2+ human induced pluripotent stem cells (hiPSCs; a gift from the Nael Nadif Kasri lab, Radboud University). That is, we employ soft-lithography and photolithography techniques to fabricate a PDMS actuator chip, containing three cell culture compartments that are connected through microtunnels. Furthermore, we demonstrate that the Ngn2-induced hiPSCs develop into neurons, both in 2D and 3D cultures, and that axons of neurons can protrude into microtunnels. In addition, we develop an approach for combining all components into one functional device to study the development of the neural network. In conclusion, we combine microtunnel devices and actuator chips to create a new microenvironment to guide neural networks and axonal growth in a predefined way and exert both static and dynamic stimuli in a specific region in the culture in a controlled way. Hence, our combined assembly is promising for studying the responses of neural networks to external mechanical forces and for the creation of more robust Brain-on-chip devices in the future.

References

[1] Xie, S. et al. (2018). *J Micromech Microeng 28*. 085011.
[2] Akcay and Luttge (2022). Introducing dynamic mechano-modulation in brain-on-chip. MPS World Summit 2022.
[3] Bastiaens, A. et al. (2020). *Front Neurosci 14*, 666

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Coupling a novel, bicompartmental MPS with a 3D, commercially available, human small intestinal tissue model to assess drug permeation and absorption

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Orally administered drug permeation and absorption across the intestinal barrier is pivotal to determine drug bioavailability. However, current *in vitro* 2D-models (Caco-2) display limited reliability. Moreover, conventional culture systems are static and do not guarantee a controlled retrieval of the biological sample, leading to potential structural alterations, affecting end-point analysis. To address this issue, we have developed TToP (True Tissue on Platform), a bicompartmental and versatile platform based on a thin cartridge able to host cell cultures, thin biopsies or organotypic tissue models, enabling a controlled retrieval of the biological sample.

Specifically, EpiIntestinal™-SMI 100 samples (EPI), a human 3D small intestinal tissue model from MatTek™ Corporation (Bratislav, Slovakia) [1] were hosted and cultured (following manufacturer's instructions) for 12 days in TToP static devices and compared with MatTek™ insert controls.

At different time points the EPI samples were fixed in 4% paraformaldehyde buffered solution and stained with Human Epithelial Antigen antibody (cell surface) and 4',6-diamidin-2-fenilindol (nuclei). Confocal microscopy (Olympus FluoVIEW FV3000RS) demonstrated a preserved tissue morphology at all time-points, complete of villo-like structures, comparable in TToP and in MatTek™ systems.

To evaluate the tightness of the tissue barrier, Trans-Epithelial Electrical Resistance (TEER) was measured at day 3, 7 and 10 of EPI culture, showing similar TEER values in TToP and in MatTek™.

In parallel, to evaluate permeability/absorption, another batch of SMI were incubated at day 10 in the presence/absence of 10 mM caffeine (2 h) and/or 100 µM Lucifer Yellow (LY) CH, Lithium Salt. Permeability determined by spectrophotometer as trans-epithelial passage of 100 µM LY from apical to basal compartment was comparable between TToP and MatTek™ systems.

At the end of cultures, samples were snap frozen or fixed, and processed for gene expression and immunofluorescence confocal microscopy (Villin, ZO-1, DAPI), showing comparable results. In conclusion, TToP demonstrated its compatibility for MatTek™ 3D *in vitro* reconstructed human intestinal model cultures, considered a standard for drug development. Permeability/absorption results support the TToP suitability for molecular absorption studies and pave the way to further applications with different tissues and/or conditions (e.g. perfusion) mimicking the *in vivo* environment.

Reference

[1] Ayehunie, S. et al. doi:10.1007/s11095-018-2362-0

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Beta cell death and IAPP: An examination through the lens of organoids on chip

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Both Type 1 and Type 2 Diabetes Mellitus (T1DM) (T2DM) have been major metabolic disorders characterized by insulin perturbation and pancreatic beta cell destruction. For T1DM, β-cell transplantation remains the most cutting-edge form of therapy but many

aspects like β-cell loss, slow vascularization and amyloid build-up hinder its success. The amyloid deposits are caused by the Islet Amyloid Polypeptide (IAPP) which acts in a behaviour similar to other amyloid forming proteins, like alpha synuclein (α-syn) and amyloid beta which are both hallmarks of neurodegenerative disorders [2]. Studies have shown that IAPP fibrils are found in pancreas of 95% of diabetic patients, especially ones with advanced forms of diabetic neuropathies and nephropathies.

There is evidence to suggest that IAPP is protected for fibrillation in secretory granules and this process is dependent on insulin [1]. We have previously shown that insulin exerts potent anti-aggregant effect on α-syn in human neuroblastoma cells SHSY5Y, primarily by activating the phospho-Akt pathway to reduce oxidative stress. Moreover, the role of IAPP in β-cell death after transplantation has not been elucidated so far because of the limitation of long-term maintenance of human islets in culture. Furthermore, 3D models of organoids represent a bridge between 2D and *in vivo* studies and can better represent physiological and pathological mechanisms that can be translated effectively.

This study is a fabrication of a microphysiological system (MPS) of β-cell (INS-1E) organoid on chip co-cultured with human umbilical vein endothelial cells (HUVEC) to model newly transplanted islet cells. INS-1E cells were overexpressed with IAPP to ascertain the effect of the protein on cell death, and particularly autophagy through mTOR. Moreover, different therapeutic targets of IAPP were also tested to estimate the reduction of IAPP fibrils.

References

[1] Guillemain, G.et al. (2022). Targeting hIAPP fibrillation: A new paradigm to prevent β-cell death? *Biochim Biophys Acta Biomembr 1864*, 84002.

[2] Kenana, A. A. et al. (2022). Linking Alzheimer's disease and type 2 diabetes: Characterization and inhibition of cytotoxic Aβ and IAPP hetero-aggregates. *Front Mol Biosci 9*.

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Developing a hiPSC-derived blood-brain barrier model to test barrier opening by microbubbles and focused ultrasound

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Alzheimer's disease is one of the most common neurodegenerative diseases worldwide, characterized by cognitive impairment. Although the disease is common and has a large impact on the quality of life, no cures are currently available. One of the primary reasons is the blockage of therapeutics by the blood-brain barrier (BBB). Microbubbles oscillating by focused ultrasound is a promising technique allowing transient BBB opening. However, the mechanisms remain elusive, partly due to differences in physiology and anatomy between lab animals and humans. Therefore, this research aims at developing a human BBB model using scalable techniques and materials for testing transient opening of the BBB for drug passage using microbubbles and focused ultrasound.

Existing astrocyte differentiation protocols were adapted and combined to differentiate astrocytes in suspension [1,2]. Astrocytes were characterized using S100B and glial fibrillary acidic protein immunofluorescence staining and functional assays on barrier integrity using transendothelial electrical resistance (TEER) and 40 kDa dextran permeation. BBB chip prototypes were fabricated using polydimethylsiloxane (PDMS) for initial validation and cyclic olefin copolymer with a polyethylene terephthalate (PET) membrane for a more scalable device.

Astrocyte spheroids showed appropriate cell survival and expression of astrocyte markers. Chip design was validated on a PDMS BBB chip and PET membranes were characterized using scanning electron microscopy and a biocompatibility assay. Using differentiated astrocytes in combination with human induced pluripotent stem cell (hiPSC) derived endothelial cells, barrier function was measured using TEER and a dextran permeability assay.

The first steps were made in developing a BBB model for evaluation of transient barrier opening by microbubbles and focused ultrasound. The devices were constructed using scalable techniques that can be implemented in industry. The protocols of hiPSCs culture and astrocyte differentiation in suspension were characterized, showing a method that can be implemented in further upscaling. Future work includes further characterization of barrier function and membrane proteins in the BBB on-chip. In addition, a novel microbubble producer and injector will be validated on

the ability to transiently break the barrier using focused ultrasound treatment.

References

[1] TCW et al. (2017). doi:10.1016/j.stemcr.2017.06.018
[2] Van der Kant et al. (2019). doi:10.1016/j.stem.2018.12.013

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Development of a 3D kidney-on-a-chip model using iPSC-derived proximal tubule cells

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Drug-induced nephrotoxicity can exacerbate acute and chronic kidney diseases and accounts for a high percentage of drug development failures in pre-clinical and clinical stages. The proximal tubule (PT) has a high expression of transport proteins and is responsible for xenobiotic clearance, which makes this part of the nephron especially susceptible to drug-induced cell damage. In pre-clinical toxicity studies, species differences can lead to poor human predictability, so the development of relevant human *in vitro* models for toxicity assessments along with the 3R's initiative is imperative. In contrast to standard 2D cell culture, 3D organ-on-a-chip models allow tubular architecture, physiological fluid flow and interaction with extracellular matrix (ECM), other tissue-specific cell types and vasculature. This mimics the native microenvironment and may improve the clinical translation of knowledge about new drugs.

In this study we differentiate human induced pluripotent stem cells (iPSC) into proximal tubular-like cells (PTL) within 14 days [1]. These cells are characterized in 2D, as well as in static transwells, and in 3D tubes under flow using the OrganoPlate® system from Mimetas. To optimize the 3D culture conditions, several seeding densities, maturation times, and ECMs or chip coatings are evaluated in the 3D system. PT-specific protein expression and cell polarization is validated by immunohistochemistry and high content imaging. Cell maturation and barrier integrity is analyzed with transepithelial electrical resistance (TEER) and fluorescent barrier permeability assays. Additionally, functional screening assays with these cells show active transport, e.g., mediated by P-glycoprotein (ABCB1), Megalin (LRP2), and other organic anion and cation transporters. To allow the comparison with similar models in the field, all results of iPSC-derived PTL are compared to a standard human proximal tubular cell line, which is commonly used for 3D models and toxicity assessments. As a further advancement of this model system, the co-culture of PTL with primary or iPSC-derived endothelial cells is under examination in all formats.

Reference

[1] Chandrasekaran, V. et al. (2021). Generation and characterization of iPSC-derived renal proximal tubule-like cells with extended stability. *Sci Rep 11*, 11575.

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Decoding chronic fatigue syndrome and long-COVID-19 using bioengineered 3-D *in vitro* skeletal muscle tissues

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Multiple studies suggest that up to 10-15% of all patients with COVID-19 may experience a range of symptoms from which some recover in 2-3 weeks, while others develop a disabling long-term sequelae picture that lasts for months or even longer after overcoming COVID-19. Long COVID-19 patients frequently report chronic fatigue syndrome (CFS) or fibromyalgia, a long-term debilitating condition compromising the patient's musculoskeletal system and bringing about severe post-exertional malaise [1]. Our research aims to understand the pathomechanism of this condition by studying the physiological and functional impacts of patient sera on skeletal muscle function using bioengineered *in vitro* 3-D platforms.

The 3-D tissues were bioengineered by encapsulating muscle precursor cells in a Matrigel-Fibrin matrix on a PDMS support. To study the effect of CFS and Long-COVID-19 sera on muscle homeostasis, mature skeletal muscle tissues were treated with patient and healthy sera. Then the tissues were characterized at structural and functional levels. On one hand, the myotube structure was analyzed by immunofluorescence techniques. On the other hand, tissues were exposed to different pulsating electric frequencies for acquiring both twitch and tetanic contractions *in situ* for functional characterization.

Preliminary comparative functional and structural analyses of tissues treated with the patient (CFS and Long COVID-19) and healthy sera after Electric Pulse Stimulatory (EPS) training suggest a significantly weaker specific force of contraction for tissues treat-

ed with patient sera. Tissues treated with CFS patient sera showed a larger myotube diameter compared to tissues treated with healthy sera. The effective cross-sectional area of the two sample sets, however, remained the same. Similar functional and structural implications of the two diseases on tissues point to a common mechanism of disease progression that appears to be metabolic in nature. The utility of this platform is a key step towards understanding patient-specific variability in disease progression and personalized response to multiple drug testing regimens.

Reference

[1] Salari, N., Khodayari, Y. and Hosseinian-Far, A. (2022). Global prevalence of chronic fatigue syndrome among long COVID-19 patients: A systematic review and meta-analysis. *BioPsychoSocial Med 16*, 21. doi:10.1186/s13030-022-00250-5

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Connecting the human intestine and liver: A primary jejunum and primary hepatocyte multi-organ MPS for more predictive studies of human drug ADME and oral bioavailability

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ADME studies are a key part of drug discovery, as the evaluation of pharmacological properties determines the efficacy and safety of a given compound. Efforts to improve the *in vitro* to *in vivo* translation of drug efficacy and safety data has led to the emergence of more complex microphysiological systems (MPS) that consist of multiple organs that are fluidically linked [1].

Here, we introduce a multi-organ MPS that links the intestine and liver using the PhysioMimix™ cell culture system, with both cell types being of primary human origin. Liver and intestinal cell lines have absent or low levels of metabolic enzyme expression, and thus fail to predict first pass human metabolism. For the intestinal barrier, crypt epithelium stem/progenitor cells were isolated from the jejunum and expanded on a biomimetic scaffold (RepliGut®). Upon differentiation, the cell layer forms a polarized barrier with a continuous layer of mucus. For the liver, primary human hepatocytes (PHH) are seeded on a 3D collagen-coated scaffold and form microtissues.

We developed a chemically defined media that supports both organs in the multi-organ MPS, and expression of metabolic enzymes and transporters was confirmed by RT-qPCR. To demonstrate improved predictive capacity, we investigated two drugs where current models fail to predict human ADME behaviour. Temocapril, which is a prodrug and is designed to be resistant to intestinal hydrolysis [2] and midazolam, which is known to undergo intestinal clearance [3].

For temocapril, we correctly observed resistance to intestinal hydrolysis by the primary jejunum model with subsequent clearance by PHHs. In contrast, Caco-2 cell carboxylesterase enzyme expression is abnormal and thus vastly overpredicts intestinal metabolism. With midazolam, we saw greater clearance when PHHs were co-cultured with a primary jejunum barrier, and this resulted in an improvement in the oral bioavailability prediction.

Here, we present a multi-organ MPS with both intestinal and liver cells from a primary human source. We demonstrate maintenance of cell functionality in co-culture and show its predictive potential for drug ADME and bioavailability studies.

References

[1] Eddington C. D. et al. (2018). *Sci Rep 8*, 4530.
[2] Imai, T. et al. (2005). *DMD 33*, 1185-1190.
[3] Jones, C. R. et al. (2016). *APPS 18*, 589-604.

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Development of an *in vitro* bile-duct-on-a-chip-platform using patient-derived cholangiocytes

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Background: Primary sclerosing cholangitis (PSC) is a disease of the bile ducts with a severe disease course without medical therapeutic options, leading to the need for liver transplantation. Cholangiocytes are the main target of destruction in PSC, yet the exact mechanisms that lead to cholangiocyte damage are not fully understood. A lack of representative disease models available to research is hindering our understanding of PSC progression. We aim to develop a physiologically relevant, robust, and simplified *in vitro* model of a human bile duct that allows us to interrogate the interactions between cholangiocytes and other disease-relevant components on a cellular level.

Methods: Chips were produced using a stereolithography 3D printer and post-cured to ensure biocompatibility. A collagen gel was placed in the central compartment with a capillary tube used to form a channel through it, and an inner coating of laminin was added to resemble the *in vivo* environment within bile ducts. Cholangiocytes were then seeded into a channel and grown for approximately 7 days before channel confluency was reached. Analysis was performed by fluorescent microscopy and biochemical assays.

Results: We developed a robust and scalable *in vitro* platform that resembles the *in vivo* architecture of a bile duct by optimizing the design and materials for high-throughput analysis, advanced microscopy, and drug screening compatibility. We included a channel in the chip, and adjusted channel size and coating to maximize physiological resemblance. The design allows incorporation of internal fluidics such as bile without the use of tubing. The chip supported growth of patient-derived cholangiocytes in a 3-dimensional gravity perfused bile-duct-like channel with strong cell-cell contacts and retained expression of key markers of cellular identity.

Conclusion: In this proof-of-concept study we provide a robust fluidic platform for culturing patient-derived cholangiocytes in a tubular structure resembling the *in vivo* bile duct architecture. The flexible chip design supports the inclusion of other disease-relevant components, such as bile or immune cells, and can be used to model various aspects of cholangiopathies *in vitro*. The use of patient-derived cells further offers possibilities for personalized drug testing approaches for the first time in a bile duct-on-a-chip model.

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Combination of cell- and tissue culture in a new fluidic flow chamber to investigate biological pathways on a physiological level

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Cells of different organs are affected by shear stress, which changes their morphology, metabolism and endocrine activity. In recent years, many cell and tissue culture experiments have been performed statically, but in order to bring them closer to *in vivo* conditions, it can be helpful to subject cells and tissue culture to a flow chamber.

To investigate biological processes affected by shear stress we developed a flow chamber, where cell and tissue culture can be exposed to fluidic shear stress. The design of the chamber allows cell seeding at a high number to obtain enough RNA and protein for qPCR and Western blot or FACS in one experimental run. It is also important to expose tissue to specific flow rates, to get a more precise understanding of what happens *in vivo*. Therefore, we developed a second loading position for placing pieces of tissue to physiological flow rates. After flow culture, apoptotic tissue can be removed, and the tissue subjected to shear rates is used for further analytical methods.

With our flow chamber we can mimic metabolic processes of intrauterine growth restriction IUGR by introducing placental villi from healthy control placentas. For this purpose, we exposed the healthy samples to a pathological flow rate, identical to patholog-

ic placentas. We got the same results of a metabolic path from placental villi from healthy pregnancies exposed to high shear rates, as those from the placental pathology IUGR. This may allow therapeutic approaches to be tested first in the flow chamber.

Due to the adaptability of the flow chamber, many different research questions can be addressed in different research fields. It will be more and more important to study molecular biological processes in a close to *in vivo* condition to get at a certain point rid of animal studies.

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A multiorgan-on-chip platform combining a tumor chamber and blood vessel for studying the intersection between type 2 diabetes mellitus and cancer metastasis

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Animal models and conventional *in vitro* cell culture methods have been used to study pathophysiology and drug metabolism in the field of cancer research for several decades. However, these models have failed to recapitulate human body's physiological and biological functions *in toto*. Organ-on-chip (OOC) models represent a breakthrough technology for biomedical research, as they promise to recapitulate human physiological and biological conditions in proximity while providing versatility by integrating different engineering challenges on their platform [1].

To date, several studies have demonstrated tumor-on-chip models along with vasculature to study angiogenesis and vasculogenesis coupled with other biological factors affecting cancer progression; However, these models are inadequate for replicating a 3D tumor microenvironment with described blood vascular system of the human body, therefore lacking to provide a suitable platform to integrate tumor microenvironment with another disease models [1].

Here we demonstrate a PDMS-based microfluidic OOC device capable of 3D *in vitro* cell culture mimicking the tumor microenvironment adjacent to a bioengineered model of vascular systems, i.e., capillary on the same horizontal plane. The latter allows ease of imaging and analysis of intercellular and cell-extracellular matrix (ECM) interactions. The model can successfully be used for the co-culture of different cell lines and mimic the stromal tumor microenvironment by supplying enough nutrients and oxygen enrichment to cells, thus preventing cell death due to artefactual hypoxia. It has been well established that cancer progression and Type II diabetes mellitus (T2DM) have epidemiological relations [2]. T2DM affects the blood vessel by affecting its structural integrity. By recapitulating the blood vascular system adjacent to the 3D tumor microenvironment, the device allows the study of tumor microenvironment interaction with a blood vessel in case of cardiovascular diseases such as atherosclerosis and diabetes. Our proposed OOC device establishes a promising tool for studying different disease models and enhances the *in vitro* drug screening model.

References

[1] Zommiti, M. et al. (2022). Organs-on-chips platforms are everywhere: A zoom on biomedical investigation. *Bioengineering 9*.
[2] Wang, M. et al. (2020). Diabetes and cancer: Epidemiological and biological links. *World J Diabetes 11*.

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Dynamic platform for continuous, high-resolution imaging of organotypic brain-tissue slices

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Organotypic slice cultures constitute a key method in research and drug testing, as tissue slices enable to preserve tissue heterogeneity and cellular organization, as well as gradients of secreted factors [1]. Organotypic brain slices are typically cultured under static conditions on porous membrane inserts or periodically exposed to medium and air environments to preserve viability [2]. However, during *ex vivo* culturing, it is not possible to have access to the tissue slices with high-resolution microscopy, which drastically limits investigations of dynamic processes. To address these limitations, we developed a membrane-based culturing approach, which can be sealed with an optically transparent layer to enable continuous high-resolution imaging.

The platform is composed of a micromilled acrylic structure featuring a microfluidic channel and a porous membrane for static culturing of the brain slices, similar to the current gold-standard culturing method. To enable high-resolution microscopy, the platform is then sealed with a glass coverslip, and the microfluidic channel is perfused with oxygenated medium to maintain slice viability during imaging. The membrane area allows for culturing of up to three cerebellar slices in parallel on each chip.

Using our platform, we were able to maintain 350 µm-thick cerebellar slices of P14 mouse pubs for 14 days under static conditions, followed by 3 days of continuous perfusion and high-resolution imaging on a spinning-disk confocal microscope. Static

culturing of the slices at the air-liquid interface enabled reorganization of the slices comparable to the existing methodology. As a proof of concept, we implanted tumor spheroids on the slices and monitored tumor-cell invasion and actin dynamics for 3 days using high-resolution microscopy. We are confident that our platform will allow for investigating dynamic processes in organotypic slice cultures of different origins, as it facilitates the controlled delivery of soluble factors and staining solutions, while enabling continuous, high-resolution imaging.

This work was financially supported by Innosuisse – the Swiss Innovation Agency (grant 46812.1 IP-LS)

References

[1] Minami, N. et al. (2017). Organotypic brain explant culture as a drug evaluation system for malignant brain tumors. *Cancer Med*.
[2] Humpel, C. (2015). Organotypic brain slice cultures: A review. *Neuroscience*.

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Development of hydrogel-based 3D *in vitro* neuronal networks

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Engineered *in vitro* neural networks are promising platforms to rapidly screen drugs and study information flow in the nervous system [1]. While existing polydimethylsiloxane (PDMS)-based microfluidic platforms offer precise architecture and connectivity, the cultured neurons grow inside microchannels on a planar multielectrode array (MEA) substrate in a two-dimensional fashion [2]. To better mimic the native extracellular matrix (ECM) microenvironment, 3D hydrogel scaffolds can be designed so that encapsulated cells can be expected to exhibit more physiological behavior [3]. Here, we propose a hybrid approach by filling the PDMS microfluidics with hydrogels to offer both a controlled topology and a physiologically relevant microenvironment to the neuronal culture. First, various hydrogels, including those based on polyethylene glycol functionalized with norbornene (PEG-NB) and gelatin methacryloyl (GelMA), were used to grow neuronal networks in 3D. These materials were further engineered by incorporating ECM components and/or tuning their mechanical properties to match the native niche environment and were able to support the growth of primary rat cortical neurons for more than 6 weeks. Next, microchannels were filled with the hydrogels to enable topologically defined growth of neurites in 3D. By tuning the hydrogel type and its physico-mechanical properties, the hydrogel-filled microstructures could be tailored for specific cell types, while the underlying MEA allows for the recording of neuronal activity over time. This platform could offer a promising tool to study cell-cell interactions in co-culture systems of neuron and glial cells, and to efficiently test the potential of various drugs in a more translational manner.

References

[1] Aebersold, M. J., Dermutz, H., Forro, C. et al. (2016). "Brains on a chip": Towards engineered neural networks. *Trends Anal Chem 78*, 60-69.
[2] Forró, C., Thompson-Steckel, G., Weaver, S. et al. (2018). Modular microstructure design to build neuronal networks of defined functional connectivity. *Biosens Bioelectron 122*, 75-87.
[3] Huh, D., Hamilton, G. A. and Ingber, D., E. (2011). From 3D cell culture to organs-on-chip. *Trends Cell Biol 21*, 745-754.

Presentation: Poster

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Communication is key: Exploring local and systemic inflammatory responses to infection using a multi-organ lung-liver-immune axis microphysiological system

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Organ crosstalk is required for communication between distant organs to maintain homeostasis. However, prolonged inflammation can result in excessive release of inflammatory markers and ultimately inter-organ dysfunction or disease. Pneumonia or sepsis from severe pulmonary infections cause hepatic acute-phase response (APR) which is largely induced by IL-6 signalling [1]. The cytokine storm associated with COVID-19 disease in particular is characterised by high expression of TNFα and IL-6 which has been shown to negatively impact liver function [2].

A multi-organ MPS was developed to model the lung-liver-immune axis to determine the effects of systemic inflammation during local lung infection by SARS-CoV-2. The CN Bio PhysioMimix™ multi-organ system was used to coculture alveolar and liver MPS with circulating monocytes. Local infection at the alveolar MPS was carried out using pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein. After 24 h, the micropumps connecting organs were activated and inflammatory, cell health and functionality markers measured over a further 48 h.

Inflammatory markers including IL-6, TNF-α and IP-10 were determined to peak after local infection. After connection of the organs, two larger peaks of inflammatory response were detected.

This demonstration of inflammatory crosstalk between the two organs corresponded with alterations in tissue health (LDH, TEER), liver function (CYP, albumin) and production of APR proteins such as C-reactive protein (CRP). Furthermore, upon addition of monocytes there was enhancement of the systemic inflammatory response, demonstrating the important role of immune cells within the system. Together, this multi-organ system allows novel and data-rich insights into both local and systemic response to infection. Greater understanding of pathogens' interaction with the body can be elucidated, allowing for more rapid and targeted drug development approaches in the future to reduce severe disease and death from both current and future novel pathogens.

References

[1] Strnad et al. (2017). doi:10.1038/nrgastro.2016.168

[2] Taneva et al. (2021). doi:10.4254/wjh.v13.i12.2005

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Organ-on-chip device integration and biological evaluation inside the Smart Multi-Well Plate

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The Smart Multi-Well Plate (SMWP), an open technology platform for Organ-on-Chip (OoC) technology developed as part of the Moore4Medical (M4M) consortium, aims to showcase the advantages of standardization in design, manufacturing and assembly for OoC [1]. In previously presented work [2], we showed integration and characterization of piezoelectric micropumps for in-line perfusion of OoC devices. Here we present the integration and preliminary biological evaluation of three OoC devices in a SMWP prototype.

This prototype, a downscaled version of the full SMWP, is constructed using stacked, predefined layers. The reservoirs of a 96-well plate are fluidically connected to integrated OoC devices and micropumps through a fluidic circuit board (FCB). A printed circuit board, assembled below the FCB, enables the electrical interfacing. The following devices were integrated in the prototype: OoC devices from Bi/ond and BEOnChip, a microelectrode array (MEA) device from MultiChannel Systems, and piezoelectric micropumps from Fraunhofer EMFT. In the OoC devices, cell culture was performed on integrated on-chip membranes. On the MEA, neuronal cells were cultured directly on the surface of the chip. For initial experiments, static cultures were performed to investigate the biocompatibility of all included materials inside the prototype.

BEOnChip performed a static cell culture with skin cells (HaCaT) in their device. Normal cell viability and morphology was observed after 96 h and 21 days of culture using Calcein-AM/PI live/dead staining. MCS performed static cell culture using hiPSCs-derived neurons directly cultured on the PLO/laminin-coated MEA chips. After 14 days of culture, eGFP staining showed normal cell morphology and network formation. Bi/ond performed an endothelial cell culture (HMEC-1), showing proper cell adhesion and viability in their devices.

The biological experiments under static conditions showed normal cell morphology and viability in all integrated devices. In the next phase of the project, the full SMWP platform with integrated perfusion will be used for biological experimentation to generate an air-liquid interface with skin cells (BEOnChip), a perfusable MEA (MCS) and endothelial tube formation under unidirectional flow (Bi/ond).

References

[1] Mastrangeli et al. (2019). *ALTEX 36*, 650-668. doi:10.14573/altex.1908271

[2] de Wagenaar et al. (2022). EUROoCS22, Grenoble (FR), 4-5 July 2022.

Presentation: Poster

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Molecular-sensitive imaging enables *in situ* monitoring of cellular dynamics at spatial and temporal resolution

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3D tissue models in Organ-on-a-Chip (OoC) systems enable to recapitulate (patho-) physiological and dynamic cellular processes such as metabolic response, phenotypic switching or tissue mechanobiology. To unravel the complex information provided by microphysiological tissue models, there is a high demand for the development of novel sensors and methods which allow for on-chip measurements.

Non-invasive imaging techniques such as Raman microspectroscopy (RMS) and fluorescence lifetime imaging microscopy (FLIM) are promising tools for marker-independent *in situ* monitoring while maintaining spatial information. Whereas FLIM targets the endogenous fluorophores NADH and FAD and is especially sensitive to metabolic changes such as oxidative stress, glycolysis or apoptosis, RMS can access various cell and tissue structures due to their unique molecular-sensitive spectral features.

We implemented RMS and FLIM for OoC setups. It was demonstrated, that RMS enables the identification and visualization of major subcellular structures e.g., nuclei, proteins, lipids. In addition to quantitative image-based assessment, analysis of the extracted spectral information can further identify alterations in molecular composition. In our models, changes in lipid composition and oxidation were demonstrated upon culture duration or external stimulation. Besides analysis of tissue aggregates RMS was further implemented for phenotyping of single circulating cells as well as for metabolites (e.g. lactate and glucose) in the surrounding medium. Moreover, FLIM enabled to investigate the metabolic equilibrium between glycolysis and oxidative phosphorylation in tissue spheroids and was sensitive to detect early signs of hypoxia and drug treatment induced apoptosis.

Overall, our results showed that both, RMS and FLIM, provide real-time insights on tissue dynamics and should be further established and developed as complementary tools to off-line and endpoint readouts in microphysiological systems.

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Glioblastoma patient-derived organoids: Characterizing the effect of tumor treating fields on the immune cell microenvironment

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Glioblastoma (GBM) is the most common and malignant primary brain tumor in adults. The current standard of care (SOC) includes maximum-safe surgical resection, radiation, and alkylating chemotherapy. Even with this SOC, the mean overall survival is only approximately 15 months, demonstrating the urgent need for more effective treatment options. While immunotherapies have made immense strides in cancer treatments, due to the low mutational burden of GBM and other factors such as an immune-suppressive tumor microenvironment (TME) and the immune privilege of the central nervous system, they have largely failed in the field of neuro-oncology [1]. Tumor Treating Fields (TTFields) therapy is an innovative treatment modality that has garnered success in treating various cancer entities over the past two decades. It is the only new treatment modality for GBM supported by a positive Phase 3 clinical trial since the introduction of temozolomide chemotherapy in 2005. Using alternating currents, TTFields create a dipole moment in certain molecules, hindering their movement and thereby preventing microtubule formation, disrupting mitotic spindle formation and the cell division necessary for proliferating tumor cells [2]. TTFields have also been shown to produce a pro-immunogenic effect, which is the rationale for our interest in the effects of TTFields on the antigenic landscape and immune TME. A major drawback in GBM research is the lack of a meaningful disease model. Our novel approach offers a new method to model GBM using patient-derived organoids to assess the efficacy of TTFields therapy *in vitro*. Using a patient-derived organoid protocol established by Jacob et al. published in *Cell* 2020, we have combined this model with Novocure's Inovitro system to administer TTFields therapy to organoids. Using organoids as a powerful tool, we plan to characterize the molecular mechanisms associated with TTFields therapy, with a focus on immune-mediated processes.

References

[1] Bhowmik, Khan and Ghosh (2015). *Biomed Res Int*.
[2] Rominiyi et al. (2021). *Br J Cancer*.

Presentation: Poster

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Development of epidermis-on-a-chip for toxicological evaluation of nanomaterials

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The use of nanomaterials in the cosmetic industry is growing exponentially due to their unique physicochemical properties. Sunscreen products containing UV nano-filters, nano-TiO2, and nano-ZnO particles can offer an advantage over their traditional counterparts owing to their broad UV protection and non-cutaneous side effects. Currently, the safety assessment of cosmetic nanoformulations uses 2D skin cell monolayers and commercially available 3D skin models cultured in static conditions that make them unable to accurately represent normal human physiology. Besides that, European legislation recently banned the use of animal testing for cosmetic ingredients. Consequently, there is a strong need for cosmetic, pharmacological, and toxicological sciences to replace those methods with reliable, reproducible, and high-throughput alternative approaches. Organ-on-a-chip technology is filled with engineering and biological challenges, but it

has the potential to revolutionize the next-generation risk assessment of nanomaterials. The goal of this work was to establish an epidermis-on-a-chip model that can simulate the complex microenvironment of the external layers of the skin to become an ideal tool for cosmetic nanoformulations screening and toxicity evaluation. The barrier integrity and cellular viability of the developed models were evaluated by TEER measurements and PrestoBlue™ assay, respectively. After culturing at the air-liquid interface, the epidermis-on-a-chip demonstrated histological features like those observed in normal human epidermis such as a proliferating basal layer and differentiating spinous, granular, and cornified layers. Further immunofluorescence analysis also indicated typical keratin expression including keratin-14, keratin-10, and loricrin. Results demonstrate that we successfully constructed a viable epidermis reliable for the future safety assessment of nanomaterials used in cosmetic formulations.

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Rapid prototyping of microfluidic co-culture platform based on a 3D printing workflow for systematic investigation of tumor stromal interactions

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Cancer-associated fibroblasts (CAFs) significantly affect the tumor microenvironment by facilitating extracellular matrix remodeling, crosstalk between different cell types, secretion of growth factors [1], and providing immune evasion of cancer cells [2]. Due to their major role in modulating the tumor microenvironment during cancer progression, CAFs are considered a prime target for cancer therapeutics. Therefore, an in depth understanding of the interactions between CAFs and cancer cells under physiologically relevant settings is highly desirable. While there has been significant progress towards use of 3D microfluidic co-culture systems towards this goal, reliance on costly and labor-intensive photolithography-based cleanroom workflows for manufacture of such research-grade complex culture systems has been a major limiting factor for rapid iteration of device design and fast acquisition of the associated biological insight [3]. To address this problem, a microfluidic multi-compartment tumor stromal co-culture platform is developed by an optimized stereolithography (SLA)-based replica molding workflow where PDMS elastomer devices are prototyped by casting against 3D printed molds. Fluorescently labeled breast cancer cells (MCF-7 and MDA-MB-231) are co-cultured with labeled CAF (544 CAF) or normal human mammary fibroblasts (HFMU 19) under 2D and 3D culture settings in reconstituted ECM in the presence or absence of interstitial flow. The device geometry is parametrized, and culture compartments are partitioned to allow transport of biochemical factors while gradually limiting mechanical interactions between different cell types. Cancer cell morphology and dynamic migratory response under varying co-culture conditions are characterized by fluorescence microscopy. Also, how the migratory behavior of cancer cells change when SF-1 (stromal derived factor 1) from CAFs inhibited, is investigated. Overall, this work demonstrates the feasibility of rapid prototyping of a series of complex microfluidic multi-compartment culture platforms based on SLA 3D printing and their application towards *in vitro* investigation of cancer-CAF interactions under varying tumor microenvironmental conditions.

References

[1] Sahai, E. et al. (2020). *Nat Rev Cancer 20*, 174-186.
[2] Hilmi, M. et al. (2020). *Cancers (Basel) 12*, 2969.
[3] Nielsen, A. V. et al. (2020). *Annu Rev Anal Chem 13*, 45-65.

Presentation: Poster

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Analysis of endothelial barrier function and polarity in 3D microvascular networks

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The endothelium is a monolayer of endothelial cells (EC) that covers the inside of all our blood vessels. The endothelium is a semi – permeable barrier between the blood on the apical side, and surrounding tissue on the basal side [1]. To prevent pathological endothelial dysfunction, barrier stability and permeability have to be tightly regulated. This is achieved by two major systems: I) molecular signaling cascades in EC (for example via small Rho-GTPases), that facilitate cell-cell contacts between EC; and II) intercellular crosstalk with surrounding, mural cells, such as vascular smooth muscle cells (VSMCs) and pericytes. Both the circulating blood, as well as these mural cells regulate EC function and barrier integrity through chemical and mechanical stimuli [1-3].

Our group has recently defined novel regulators of cell-cell and cell-matrix adhesion in healthy and inflamed primary human EC. We aim to show that these regulators are vital to maintain EC polarity and barrier integrity. We will address this aim in conventional 2D cell models and more complex 3D Vessel on Chip (VoC) systems that include ECs, VSMCs and/or pericytes to study EC polarity in a highly translatable environment. To further increase physiological relevance, we will work with iPSC-derived cells. This approach allows us to generate isogenic multicellular VoCs and opens up the

opportunity to include patient-derived cells that carry mutations known to perturb endothelial polarity. This way, we expect to identify novel key players that maintain endothelial apico-basal polarity and barrier integrity, which is valuable information in a translational context for CVDs that show impaired microvascular function.

References

[1] Kruger-Genge, A. et al. (2019). Vascular endothelial cell biology: An update. *Int J Mol Sci 20*.
[2] Beckers, C. M., van Hinsbergh, V. W. and van Nieuw Amerongen, G. P. (2010). Driving Rho GTPase activity in endothelial cells regulates barrier integrity. *Thromb Haemost 103*, 40-55.
[3] Mendez-Barbero, N., Gutierrez-Munoz, C. and Blanco-Colio, L. M. (2021). Cellular crosstalk between endothelial and smooth muscle cells in vascular wall remodeling. *Int J Mol Sci 22*.

Presentation: Poster

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Development of a 3D organ-on-chip model of the collecting duct for disease modelling

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Introduction: Extracellular matrix (ECM) is a network of proteins and proteoglycans essential for supporting cellular proliferation and physiological behaviour. Particularly, it was shown that kidney basement membrane components (mainly collagen IV and laminin) play a crucial role in the function of different segments of the nephron [1]. An example of this mechanism is hensin, an ECM protein that have shown to promote transition between α and β intercalated cells, stimulating different mechanisms of action in the collecting duct [2]. Current models using organ-on-chip systems mainly use collagen I as scaffold for their systems, without considering segment-specific compositions. Additionally, most kidney models reproduce proximal tubule systems, with no current 3D model of the collecting duct to study physiological mechanisms *in vitro*. Therefore, the aim of the study is to develop a 3D model of the collecting duct using organ-on-chip system by integrating more physiological and biomimetic ECM components.

Methods: Madin-Darby canine kidney cells (MDCK II) were cultured in MEM supplemented with 10% FBS and 1% Pen/Strep. Three-lane organ-on-chip systems (OrganoPlate, Mimetas) were used to produce tubular structures, where the middle channel was filled with a permeable ECM component (Collagen I, neutralised with NaOH and Hepes) and the top channels was filled with MDCK II.

Results and conclusions: Preliminary results of the culture of MDCK II on collagen I gels showed high proliferation and viability on a flat collagen gel whilst the seeding in the channel of the organ-on-chip system was less successful. Therefore, different cell lines to better represent collecting duct cellular population and further optimisation of the ECM composition is required to successfully produce and culture a full functional tubular structure. Future work includes inclusion of basement membrane proteins such as collagen IV and laminin to the collagen I. The goal of the study will be to eventually incorporate urine-derived cells from patients to create a more personalised platform for disease modelling.

References

[1] Abdollahzadeh et al. (2022). doi:10.1016/j.diff.2022.02.001
[2] Gao et al. (2010). doi:10.1073/pnas.1010364107

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Retina-on-chip: Designing a PDMS-based microfluidic chip with 2 µm-thick membranes for culture of iPSC-derived retinal pigment epithelium

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The eye is the window to the world around us. Vision is acquired through the conversion of light to an electrical signal by the retina. The human retina can be roughly divided into three sections, namely 1) the neural retina, including photoreceptor cells, 2) the retinal pigment epithelium (RPE), and 3) the choroidal vasculature which is separated from the RPE by the Bruch's membrane [1].

When retinal cells do not function properly, either due to genetic defects (inherited retinal diseases) or ageing (e.g. age-related macular degeneration) retinal degeneration occurs, leading to progressive loss of vision. To further understand disease processes and optimize new therapeutic modalities, patient-specific cell culture models will be indispensable. Organ-on-chip models of the retina have the potential to capture all three sections of the retina in a controlled, and patient-specific manner [1,2]. In this project, we aim to develop an induced-pluripotent stem cell (iPSC)-derived retina-on-chip device based on cellular structures found in the human retina. An important challenge in the development of such mod-

els is to controllably replicate the multi-layer retinal structure with scaffolds and membranes while maintaining cell-cell interactions.

Here, we demonstrate that thin synthetic membranes, that have similar thickness of the basal laminae in the human retina, can support long-term culture of iPSC-RPE monolayers inside microfluidic chips [3].

For this, a polydimethylsiloxane (PDMS)-based microfluidic device was fabricated, consisting of two layers separated by a 2 µm-thick PDMS membrane to mimic Bruch's membrane. After coating with a basement membrane matrix, iPSC-RPE were seeded, cultured, and maintained for at least 5 weeks on-chip. Due to the flexibility of the membrane, the iPSC-RPE showed the formation of a curved monolayer. Furthermore, the iPSC-RPE showed increased pigmentation over time and high expression of tight junction protein-1.

Next experiments will look into the addition and characterization of a functional vascular network underneath the thin membrane, as well as retinal organoids in the semi-curved microcompartment. When the full model is established, we will focus on creating patient-derived retinal models.

References

[1] Veleri et al., doi:10.1242/dmm.017913
[2] Achberger et al., doi:10.7554/eLife.46188
[3] Koolen et al., doi:10.1016/j.scr.2022.102670

Presentation: Poster

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Increased local testosterone levels alter human fallopian tube genetic profile and signaling on the PREDICT-MOS microfluidic platform

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Androgen plays a critical role in the physiology of the female reproductive system. In fact, androgen acts on ovarian function by modulating follicles development, growth, and survival. Increased androgen has also been associated with increased risk of ovarian cancer. High Grade Serous Ovarian Carcinoma (HGSOC), the most common and aggressive histotype of ovarian cancer, originate from the fallopian tube epithelium (FTE). Only recently, the role of testosterone on FTE function has been investigated and showed to decrease cilia function and promote proliferation of murine oviductal cells which are the equivalent of FTE cells. Recently testosterone secretion from the ovary has been shown to be augmented by co-culturing the ovary in proximity of tumorigenic FTE cells. Oral contraceptives and tubal ligation and hysterectomy which protects against ovarian cancer, decreases circulating levels of androgens. Nevertheless, the mechanism of androgen action on HGSOC tumorigenesis has not been determined.

In the current study, we investigated the effect of increased testosterone on FTE and found that testosterone upregulates WNT4 and induces migration and invasion of immortalized human fallopian tube cells. We profiled primary human fallopian tissues grown in dynamic conditions by RNA sequencing and found that p53 and its downstream target genes (PAX2, p21, and CD82) were downregulated in response to testosterone treatment. A novel microfluidic platform, the PREDICT-Multi Organ System (PREDICT-MOS) was engineered to support insert technology that allowed for the study of cancer cell migration and invasion through Matrigel when subject to dynamic flow. Using this system, we found that testosterone enhanced FTE migration and invasion, which was reversed by the androgen receptor (AR) antagonist, bicalutamide. Testosterone also enhanced FTSEC adhesion to murine ovarian stroma. Overall, these results indicate that primary human fallopian tube tissue and immortalized FTSEC respond to testosterone to shift expression of genes that regulate invasion while leveraging a new strategy to study migration in the presence of dynamic fluid flow.

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Human cortical neuron incorporation into blood-brain barrier microfluidic model for drug screening in neurodegenerative diseases

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Most of the neurodegenerative diseases (NDDs) are characterized by the degeneration of the neurons in the nervous system. Neurofilament light chain (NfL) is a promising biomarker to monitoring NDDs because is a protein expressed exclusively in neurons is only released upon neuronal damage and it is secreted in the extracellular matrix. The development of *in vitro* models with human neurons could offer a reliable platform to assess the drug's crossing to the blood-brain barrier (BBB) and its performance against neurodegeneration once inside the brain. In this work, we incorporate cortical neurons from embryonic stem cells (ESiN) in a BBB microfluidic device. ESiN are embedded in a 3D scaffold with other cells that play a key role in their development as astrocytes and pericytes. Also, brain endothelial cells were included to mimicking the BBB which protect the entrance of external components as drugs to the central nervous system. Cell viability of all the cells included in the device was evaluated using different cell media combinations.

Also, neuronal differentiation into the chip was determined using markers as NeuN and NfL. Neuronal damage was modelled in the chip by excitotoxicity using N-methyl-D-aspartate (NMDA) thus promoting NfL release by axonal degeneration. Finally, we expect to evaluate the drug performing against neurodegeneration using our microfluidic device with ESiN through NfL monitoring.

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Simultaneous induction of vasculature and neuronal network formation on a chip reveals a dynamic interrelationship between cell types

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While the vascular network provides oxygen, nutrients, and signaling molecules to tissues, the neuronal networks receive, process, and deliver information to regulate bodily functions. Thus, these two form vital network systems in the human body, which interplay physically and functionally with one another. These neurovascular interactions are vital for tissue development and maintaining homeostasis. There is a need for more relevant neurovascular *in vitro* models to study these interactions in more detail. The current used models are typically established as short-term (≤ 7 days) cultures, and they lack the supporting vascular mural cells [1].

Here, human induced pluripotent stem cell (hiPSC)-derived neurons [2], fluorescence tagged human umbilical vein endothelial cells (HUVECs), and either human bone marrow or adipose stem/stromal cells (BMSCs or ASCs) as the mural cell types [3] were utilized to create a novel 3D neurovascular networks-on-chip model. Collagen-fibrin hydrogel was used to establish long-term (≥ 14 days) culture in a commercial chip proving a proper microphysiological environment [3].

Medium optimization revealed that aprotinin-supplemented endothelial cell growth medium-2 (EGM-2) supported the simultaneous formation of neurovascular networks, mural cell properties, and the stability of the hydrogel. Both neuronal and vascular networks were also morphologically and functionally characterized. According to results, vasculature formation was supported by the neuronal networks by establishment of direct cell-to-cell contacts and drastic increase in secretion of angiogenesis-related factors in contrast to vasculature cultures without neurons. Both mural cell types supported the formation of neurovascular networks; however, the BMSCs seemed to enhance the neurovascular networks in greater extent.

Taken together, we present a novel human neurovascular networks-on-chip platform that is applicable for creating *in vivo*-like tissue model with intrinsic crosstalk between the cell types. This 3D neurovascular network model forms an initial tool for developing vascularized and innervated organ-on-chip and further body-on-chip concepts and offers the possibility for mechanistic studies on neurovascular interactions.

References

[1] Osaki, T., Sivathanu, V. and Kamm, R. D. (2018). *Sci Rep 8*, 1.
[2] Hyvärinen, T., Hyysalo, A. et al. (2019). *Sci Rep 9*, 1.
[3] Mykuliak, A., Yrjänäinen, A. et al. (2022). *Front Bioeng Biotechnol 10*, 1.

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Development of advanced setups with integrated readouts for evaluation of cardiotoxicity in a heart on chip device

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Organs-on-Chip (OoC) have recently emerged as promising tools to generate advanced cardiac *in vitro* models, enabling to recapitulate key physiological cues of the native myocardium and to provide a means to directly analyze functional parameters.

In this work a microfluidic platform (uHeart; [1]) was exploited to generate 3D cardiac models from different cell sources, such as chick embryo cardiomyocytes (CMs) isolated from primary tissue and human iPSC-CMs mixed with cardiac fibroblasts, which were then developed by exploiting different stimulation set-ups (i.e., mechanical and/or electrical stimulation). uHeart enabled to analyze 3D cardiac microtissues through combined readouts (e.g., video acquisition and electric field recording [2]. A comparison between uHeart and commercially available MEA systems, the gold standard for 2D cardiac electrophysiology was performed by evaluating functional parameters (e.g. viability, spontaneous beating onset time, synchronicity, electrophysiological parameters).

Finally, a drug screening was carried out to preliminarily assess the ability of uHeart to be used as a predictor of cardiotoxicity, such as MEA systems.

A combination of architectural (i.e. three-dimensionality and higher cell density), biophysical (i.e. mechanical and electrical stimulation) and environmental cues was demonstrated to be fundamental to elicit an early onset of spontaneous beating and a higher viability after a prolonged culture in uHeart as compared to MEA. A combination of video, calcium transient analyses and field potential recordings allowed to determine that 3D stimulated microtissues have higher capability to get faster synchronous beating with respect to both 2D and 3D static samples. In particular, the mechanically actuated samples emerged as the most capable to keep a stable pace of CM, while both mechanical and electrical stimulation allowed to induce a precise pace after some days of training. Furthermore, uHeart was able to determine properly both negative and positive chronotropic effect of different drugs, such as E4031 and Isoprenaline, respectively.

In conclusion, this work highlighted the capability of uHeart to develop functional cardiac models and to successfully predict different drug-induced alterations, qualifying as promising cardiotoxicity screening tool.

Reference

[1] Visone et al. (2018). *APL Bioengineering.*
[2] Visone et al. (2021). *Biofabrication*.

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Development of a liver-heart multi-organs-on-chip platform for drug toxicity studies

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The drug development pipeline (DDP) is a costly and time-consuming process, characterized by high failure rate due to unpredicted harmful side-effects. Undetected hepatic and cardiac toxicities are the main causes of safety-related drug failures throughout the entire DDP and during post-market surveillance [1]. To address this issue, Multi Organs-on-Chip (MOoC) have recently been developed as innovative tools for more predictable and effective preclinical models. A liver-heart MOoC platform (LivHeart) was developed to predict cardiotoxic side-effects of drugs after the hepatic metabolism. The LivHeart was designed to host in the same platform the MPCC liver-on-chip [2] and the beating heart-on-chip, integrated with a system of electrodes to directly monitor the electrophysiology of the cardiac model [3]. Systems of normally-closed microfluidic valves were integrated into the platform to finely control the communication and the diffusion between the models, and to avoid convection due to possible hydrostatic pressure differences. The working principle of the valve, the mechanical stimulation on the cardiac construct and the diffusion profile were characterized either experimentally and/or with a numerical simulation. For the drug toxicity investigation, the platform was subjected to Terfenadine (TER), a cardiotoxic compound that is metabolized by the hepatic enzymes into its non-cardiotoxic form Fexofenadine (FEX). The results obtained by monitoring drug-induced alterations in cardiac viability and electrical activity proved the functionality of the platform to screen the effect of pro- (i.e., the TER-induced prolongation of the depolarization-repolarization interval of cardiac electrical signal) and metabolized- (i.e., no alteration of the electrophysiological parameters following the presence of FEX) drugs. The effective metabolism of the drug was further confirmed by the percentage of FEX present in the liver compartment after the TER incubation (i.e., 90% vs 10%), measured by Multiple Reaction Monitoring (MRM) mass spectrometry. The LivHeart platform was proved able to predict off-target cardiotoxicity in a more physiological manner than other available *in vitro* models, providing a further example of the advancements that MOoC can bring to the drug safety testing in the preclinical phases of DDP.

References

[1] Ferrari and Rasponi (2021). *APL Bioeng*.
[2] Ferrari et al. (2021). *Biomed Mater*.
[3] Visone et al. (2021). *Biofabrication*.

Presentation: Poster

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Bioluminescence imaging of microfluidic chips for continuous, non-invasive, and *in-situ* bio-screening

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The integration of optical biosensors in microfluidic organ-on-chip platforms is urgently needed to monitor cellular and molecular processes in real-time. Although fluorescence imaging (FLi) is the preferred method, it is not always suitable for *in situ* evaluation and

non-invasive real-time screenings. Recently, developments in bioluminescence imaging (BLi) allow to detect biological readouts with high sensitivity and signal-to-noise ratio [1]. Here, we report the integration and optimization of BLi in organ-on-chips for single-cell imaging, using a poly(dimethylsiloxane) (PDMS) chip (1.7 x 1.2 x 0.4 cm) with a central cell chamber for optical detection (7.5 x 2 x 0.25 mm). In short, HEK-293T cells were engineered to express NanoLuc® luciferase and GFP, integrated on an on-chip platform, encapsulated in a distinct ECM-like hydrogels (Fibrin gel, GelMA and Agarose), to prove extended compatibility of this technology with distinct 3D matrixes. Mechanical properties and diffusion rates were assessed using nanoindentation and diffusion quantification was done using fluorophores within the molecular weight interval of the BLi substrate (0.3 kDa). All the hydrogels allowed 100% diffusion in the first 20 minutes. After encapsulation and integration on-chip, cells presented viability of > 90% after one day of culture. BLi photon flux of cell-laden gels comprised of 3 x 10^5 HEK-NanoLuc cells integrated on-chip was quantified after furimazine (20 µM) injection at IVIS imager spectrum (Perkin Elmer) (≈ 8,5 x 10^6 photons/sec). We then used a Zeiss Elyra PS1 SIM microscope installed with a sensitive EMCCD camera (Andor iXon DU 897, 512 x 512 pixels) to BL or FL emitted by the on-chip integrated HEK-NanoLuc cells. Simultaneous multicolor optical imaging of single cells on-chip (≈ 2.4 x 10^6 counts/sec) was achieved for 20 minutes post injection of Fumirazine substrate (infusion rate ~20 µl/min). BL photons registered ≈ 2-fold greater signal-to-noise ratio compared to GFP emissions. In summary, integration of BLi on the organs-on-chips can allow sensitive real-time imaging of multicolored cells in a non-invasive manner. Overall, this work presents the first-of-its-kind integration of multiple optical biosensor read-outs for organ-on-chip platforms. Additionally, the incorporation of BLi will allow real-time and on-site monitoring of biological events at a single-cell level.

Reference

[1] Moreira Teixeira, L. and Mezzanotte, L. (2021). *View 2*.

Presentation: Oral

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Utilizing commercially available automated stacking machines to scale organ-on-chip manufacturing from prototype to volume production

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Organ-on-a-Chip (OoC) technology is gaining importance as novel *in vitro* models are being developed to meet the requirements of research and industry. As a result, a plethora of different microfluidic layouts have been presented. However, most of the OoC-platforms are limited by the mere fact of scalability in production, making it challenging to moving these from laboratory to industrial scale fabrication. Due to the ease of implementation, stacking multiple structured layers to a complex device is an increasingly common fabrication method. In this manufacturing process, individual polymer layers are first structured and functionalized, and then manually aligned and joined to form an integrated system. In mass production of microfluidic systems, integration, assembly, and interconnection processes account for up to 80% of the total manufacturing cost [1]. We here present a novel method for automated stacking of polymer films by adopting equipment from low-temperature co-fired ceramic (LTCC) technology. Thus, we take an existing prototyping manufacturing process and scale it up using established production processes and equipment, creating a volume-independent process chain. In this way, the design and material remain identical throughout the scaling process, ensuring a seamless transition from prototype to product.

To demonstrate the functionality of our technology, we translated the lab-scale layer-by-layer manufacturing process used to fabricate the recently published pump-less recirculation Organ-on-a-Chip (rOoC) platform [2] to high-volume fabrication using an off-the-shelf LTCC-stacking machine. First, the layout was transferred into a format suited for the LTCC-stacker and laser-cut into thermoplastic films using a femtosecond laser. Next, the films were UV activated and finally stacked using the semiautomatic Stacker from Keko (Model ST-2MMV). In the final step, a CO2-laser-cut reservoir plate was thermally bonded to the stack. To show proof-of-concept, we seeded human umbilical vein endothelial cells (HUVECs) into the channels and cultivated the cells for several days under gravity-driven perfusion. Cells aligned similar

to the published results, but using the novel fabrication step we could speed-up fabrication time while ensuring a positioning accuracy of ± 10 µm.

References

[1] Clerc, S. (2020). Status of the Microfluidics Industry 2020.

[2] Busek, M. et al. (2023). Pump-less, recirculating organ-on-a-chip (rOoC) platform. *Lab Chip.*

Presentation: Poster

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Beyond mobile phone displays: Leveraging flat panel display technology for biomedical applications

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Microphysiological systems, including organoids, 3-D printed tissues and organ-on-chips (OoCs), are physiologically relevant *in vitro* models that have experienced explosive growth in recent years. OoCs combine biology with microfabrication to mimic key aspects of human physiology and diseases. They have emerged as a human-specific experimental platform for preclinical research and therapeutics testing that will reduce the cost of pre-clinical drug development, provide better physiological relevance and replace animal testing. Yet, the lack of standardization and cost-effective fabrication technologies hampers wide-spread adoption of OoCs. In this presentation, we will highlight our work on the use of scalable flat panel display (FPD) technology as an enabling and cost-effective technology platform for biomedical applications by demonstrating facile integration of microfluidics and microelectronics in the standardized 96-well plate format.

Flat panel displays are ubiquitous in our daily lives and used in mobile phones, laptop computers, tablets and TVs. The manufacturing technology relies on large glass substrates and cost reduction is enabled by increasing substrate size. Nowadays, mobile phone displays are fabricated on "GEN6-size" (1.5 x 1.9 m^2) and high-end TVs on "GEN10.5-size" (2.9 x 3.4 m^2). Using the same technology, we have developed a range of individual and integrated OoC modules, i.e. metal electrode arrays, microfluidics, electronic valves, flow sensors and optical sensors and integrated those in the 96-well plate format. Materials and processes used are FPD compatible, thus enabling reliable manufacturing at industrial scale.

Individual and integrated modules were tested for their biological applicability in OoCs. Besides direct cytotoxicity, biocompatibility and material adsorption has been evaluated. Direct cytotoxicity was performed according to ISO (10993-5) standards and showed no direct cytotoxicity of any of the tested materials. Biocompatibility was tested by culturing relevant cells and cell lines in direct and indirect contact with the materials, including functional read-outs such as cell viability (neutral red, MTT), cell functionality (epithelial barrier properties: FITC-dextran and lucifer yellow permeability) and cell proliferation. Adsorption, a key aspect especially when applying OoC models for pharmaceutical application, was tested by performing mass balance studies with (radiolabeled)-test compounds (e.g. atenolol, antipyrine, caffeine, mannitol, warfarin).

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Studying the effect of ischemia on tissues – A research overview of the Centre of Excellence in Body-on-Chip research (CoEBoC)

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Background: CoEBoC aims to develop body-on-chip (BoC) systems to study ischemia-related pathologies which are leading causes of diseases with high mortality and morbidity rates worldwide. We combine multidisciplinary expertise in human stem cells, biomaterials, sensors, microsystems and biomodelling and -imaging as well as clinical knowledge. The aim is to develop multitissue models for studying tissue interactions when exposed to hypoxic conditions. The focus is on developing brain, heart, liver, and adipose tissue models, all integrated with vascular structures and neural connections (innervation). These form the key tissues of ischemic injury and are key players to understand ischemia-related pathophysiologies.

Methods and technologies: Various differentiation techniques for human-induced pluripotent stem cells (hiPSCs) and adult stem cells are developed. The oxygen content is regulated and monitored in the BoCs. Biofunctionalized hydrogels withstanding hypoxia are developed to support and control the cell behavior in 3D. Several sensing and imaging technologies are integrated into the BoCs including electrophysiology, bioimpedance, and optics-based measurements with the focus in 3D applications. Computational *in silico* models of cellular functions are developed.

Recent developments: We have formed several significant tissue and patient models in 2D, organ-on-chip, and 3D formats and de-

veloped acute and chronic hypoxia chips including oxygen sensing. We can gain spatiotemporal control of oxygen with the possibility to change the oxygen content in a few minutes [1]. In a model for ischemic heart disease, a heart failure drug was tested on hiPSC-cardiomyocytes during and after hypoxic stress and the antiarrhythmic effect of the drug was clearly demonstrated [2]. Further, a computational metabolite-sensitive model of hiPSC-CMs electromechanics was used for predicting the effects of three drugs on molecular mechanisms of contraction [3]. We have developed human cell-based and electrophysiologically functional cardiac innervation on a chip in a compartmentalized microfluidic device [4], Neuronal and vascular 3D models and their computational 3D construction is under vigorous development [5,6] A gellan gum hydrogel for regulating vascular guidance was developed [7].

References

[1] Tornberg (2022). doi:10.1007/s10544-022-00634-y
[2] Gaballah (2022). doi:10.3390/cells11061045
[3] Forouzandehmehr (2022). doi:10.3389/fphys.2022.1010786
[4] Häkli (2022). doi:10.3390/ijms23063148
[5] Harju (2022). doi:10.1242/dev.200012
[6] Mykuliak and Yrjänäinen (2022). doi:10.3389/fbioe.2022.764237
[7] Gering (2022). doi:10.1016/j.bioadv.2022.213185

Presentation: Poster

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Integrated oxygen and TEER sensing enable rapid kidney toxicity detection in a high-throughput co-culture of the human proximal tubule

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Microphysiological systems (MPS) are rapidly improving the ability to generate physiologically relevant tissues at drug development scales, but there remains a need for methods to efficiently monitor tissue responses in complex systems. We have recently developed a high-throughput MPS with integrated oxygen and TEER sensing for near-real-time readouts of metabolic health and barrier function. To implement this system in drug development workflows, it is critical to understand how these readouts can be applied to evaluate toxicity in specific tissue types. Here, we demonstrate the potential of optical-based oxygen sensing and rapid TEER measurement in the PREDICT96 (P96) high-throughput organ-on-chip platform for investigating acute and repeated toxicity in a human co-culture model of the kidney proximal tubule.

Human primary renal proximal tubule epithelial cells (hRPTEC) were co-cultured with human primary microvascular endothelial cells under unidirectional flow in the P96 microfluidic culture plate consisting of 96 bilayer devices with integrated oxygen and TEER sensors. Tissues were exposed to doses of cisplatin, a well-characterized kidney-toxic drug, between 1 and 200 µM for up to 5 days. Oxygen consumption and TEER were measured daily to identify changes in metabolic health and barrier function and were validated against immunofluorescent imaging and a standard lactate dehydrogenase (LDH) toxicity assay.

Both oxygen consumption and TEER readouts demonstrated dose and time-dependent changes in tissue health. Oxygen readouts enabled prediction of a toxic threshold for cisplatin, which decreased exponentially from 19.8 µM at 1 day to 2.3 µM after 5 days of dosing. Consistent with the predicted threshold, TEER confirmed a significant reduction in barrier function within 1 day of exposure to 25 µM which did not occur until day 3 for 15 µM. Changes in TEER correlated with hRPTEC tight junction expression, and both oxygen and TEER aligned with cell death profiles indicated by LDH release.

Oxygen consumption and TEER sensing revealed dynamic toxicity responses of kidney tissues with rapid, non-invasive data acquisition. This study demonstrates that MPS enabled by multi-parametric sensing approaches have the potential to improve the efficiency of drug development pipelines.

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Compartmentalized structure for hypoxia and control of oxygen microenvironments with good spatiotemporal precision

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Organs with restricted blood flow become hypoxic as oxygen supply is decreased. The affected tissue site experiences local hypoxia, which is not a homogenous environment for the cells. Generally, the microenvironments can be divided into three regions. The core forms an anoxic region, with most restricted blood flow and no oxygen. A hypoxic region with clearly decreased oxygen levels forms around the core. The tissue surrounding the hypoxic region is normoxic with normal vasculature and oxygen levels. Each region may contain multiple cell types that respond differently depending on their oxygenation status.

We have developed a microfluidic channel structure [1] that is combined with a coculture device [2], allowing cell compartmen-

talization by cell type or oxygen concentration. The core of the device is the microfluidic channel structure consisting of three separate compartments. Each compartment contains an array of microchannels positioned beneath cell culture compartments and separated by a 20 µm thick membrane. The used material is polydimethylsiloxane (PDMS). We characterized the oxygen modulation dynamics and spatial resolution using a 2D ratiometric oxygen imaging system. We also demonstrated the membrane being a suitable cell culture substrate after fibronectin coating, utilizing commercially available live/dead staining kit.

The developed structure was capable of creating different oxygen profiles from normoxic (19% oxygen) to hypoxic (0% oxygen). Despite showing some spatial differences in the dynamics, the structure is capable of modulating the oxygen concentration within minutes representing time scale that is relevant for stroke or heart attack. Combining the microfluidic channel structure with the coculture device, different oxygen profiles can be created simultaneously, while cells stay connected via microtunnels. The structure can create distinct oxygen profiles simultaneously in dedicated compartments, with minimal interference to each other, despite the permeable nature of PDMS and the microtunnels. As live/dead staining show the structure being suitable for cell culture, we have moved forward to incorporate coatings needed to model brain and cardiac infarcts. The device provides precisely controlled oxygen environments to study cell responses.

References

[1] Tornberg, K. et al. (2022). *Biomed Microdevices 24*.
[2] Ristola, M. et al. (2019). *J Micromech Microeng 29*.

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The generation of conjoined 3D vasculatures within a novel barrier-free, open top microfluidic chip for multi-tissue modelling

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Microfluidic chips designed for recapitulating complex 3D tissues are a significant advancement from the traditional 2D cell cultures. Currently, many microfluidic chips utilize a certain design: a lateral gel channel for hydrogel-embedded cells flanked by two media channels allowing the generation of fluid flow across the hydrogel area. Still, establishing multicellular tissue models requires pre-determined cell compartments for sustaining tissue-like interactions [1]. Here, we introduce our novel barrier-free chip design with a direct opening onto a gel channel implementing another cell culture area. We demonstrate the formation of conjoined 3D vasculatures and assess the morphology of the vasculatures in terms of vascular volumes and interconnectivity between the networks.

Conjoined vascular networks were generated in a stepwise manner. First, fibrin-embedded GFP-tagged Human Umbilical Vein Endothelial Cells (GFP-HUVECs) and human Adipose Stem/Stromal Cells (ASCs), 5 million/ml and 1 million/ml, respectively, were mixed and transferred to the gel channel and let to gelate (15 min) [2]. Next, fibrin-embedded RFP-HUVECs and ASCs were similarly mixed and transferred to the opening on top of the gel channel and let to gelate (10 min). Three gravity-based flow conditions were established: "up-to-down", "side-to-side" and "static". Flow conditions were re-established daily for 5 days. The forming vasculatures were imaged daily (Leica DMi8). Chips were stained with phalloidin and DAPI and imaged with a laser scanning confocal microscope (Nikon A1R) to observe and quantify vascular network characteristics.

All flow conditions allowed the formation of the conjoined vascular networks supported by ASCs. We also observed morphological differences of the vascular networks between the studied flow conditions. The quantitative vascular network characteristics of the flow conditions i.e. vascular area, volume, average vessel diameter and total length, are currently investigated. Moreover, we are quantifying interconnectivity of the networks, i.e. GFP-HUVEC vessels merging with RFP-HUVEC vessels.

The described findings demonstrate that the novel, barrier-free chip design is suitable platform for establishing a vascularized multicellular culture. The opening on top the vasculature allows the addition of another tissue-specific 3D culture to be implemented in direct contact with the vascular network as a multitissue model.

References

[1] doi:10.1038/s41576-022-00466-9
[2] doi:10.3389/fbioe.2022.764237

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Modeling cardiac ischemia-reperfusion and border zone by using human induced pluripotent stem cell-derived cardiomyocytes

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Ischemic heart disease (IHD) is the most common cardiovascular disease and the leading cause of death worldwide. In IHD, the blood flow to myocardium is reduced or blocked, leading to oxygen and nutrient deprivation and accumulation of metabolic waste in the tissue. However, human based IHD models are currently lacking, and animal models might not faithfully recapitulate human disease mechanisms and responses to treatment.

We have successfully modeled ischemia-reperfusion (IR) using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and managed to recapitulate several known cellular IR responses [1,2]. With our system, hypoxia is introduced in a matter of minutes as an immediate shock to the cells, which allows evaluating the hiPSC-CM responses to IR. hiPSC-CM electrophysiology was evaluated using microelectrode arrays (MEA), while monitoring the oxygen dynamics of the culture. Based on our results, hiPSC-CM beating frequency and field potential amplitude decreased, and field potential propagation slowed down during hypoxia but recovered during reoxygenation. Furthermore, the cell morphology deteriorated especially during reoxygenation. The observed changes showed that hiPSC-CMs can recapitulate the known acute IR responses, and thus they can be used in modeling the IR disease mechanisms and treatments.

In addition to the previous cardiac ischemia on-chips, we have now included oxygen gradient to our platform to model the border zone, the area between the injured, hypoxic tissue and the adjacent viable, normoxic tissue formed to the heart after myocardial infarction. The oxygen gradient is formed with a custom-made gradient lid. The lid includes two parallel thin-wall silicone tubes providing an oxygen gradient on the area between the tubes. Depending on the design of the gradient lid the steepness of the gradient can be varied. A steady oxygen gradient was successfully created with 19% and 0% oxygen gas mixtures validated utilizing a 2D ratiometric imaging of oxygen. Transparent lid enables simultaneous gradient formation and the monitoring of the cardiac function with MEA and video microscopy.

References

[1] Häkli, M. et al. (2021). *Sci Rep 11*.
[2] Häkli, M. et al. (2022). *Stem Cells Int.*

Presentation: Poster

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Engineering a microfluidic based living neural interface towards vision restoration

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Restoring functional vision of blind people without an optic nerve requires targeted single neuron stimulation in the visual thalamus located deep inside the brain. We present an integrated stretchable microfluidics living neural interface in which we exploit real neurons as relays to convert electrical signals into spikes for synaptic stimulation of thalamic target neurons. Our platform consists of a stretchable microelectrode array [1] aligned with axon guiding PDMS based microstructures that contain living retinal neurons. To direct axons towards our target location inside the brain we unidirectionally merge all axons on the chip into a common bioresorbable nerve conduit [2].

We show that rat retinal spheroids seeded onto our device form an up to 4 mm long artificial optic nerve that can transit from the device to reinnervate a Matrigel based target structure *in vitro*. Moreover, we show how stimulation of retinal cells within our microstructures [3] enables synaptic modulation of thalamic target neuron activity *in vitro*.

Taken together, the "living component" in the neural interface has the potential to increase stimulation resolution and enables targeting of subpopulations of neurons inside the brain.

References

[1] Renz, A. F., Lee, J., Tybrandt, K. et al. (2020). Opto-E-Dura: A soft, stretchable ECoG array for multimodal, multiscale neuroscience. *Adv Healthc Mater 9*, e2000814.
[2] Madduri, S., Feldman, K., Tervoort, T. et al. (2009). Collagen nerve conduits releasing the neurotrophic factors GDNF and NGF. *J Control Release 143*, 168-174.
[3] Duru, J., Küchler, J., Ihle, S. J. et al. (2022). Engineered biological neural networks on high density CMOS microelectrode arrays. *Front Neurosci 16*, 829884.

Presentation: Poster

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Rapid prototyping ISO compatible organ-on-chip devices

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The organ-on-chip community is currently seeking to achieve large-scale adoption. Reproducibility, parallelization, multi-organ combination, sensor integration, integrated fluidic functions (pumps, valves) and moving away from PDMS are some of the main challenges in this domain. We recently developed standardized microfluidic building blocks and a Fluidic Circuit Board (FCB) that can be scaled up and allows the modular integration of the before-mentioned functionalities. The systems is ISO ISO22916:2022 [1] compatible and ready for any organ-model integration.

At CEA Leti, we have developed a set of hot-embossing moulds that allows both rapid prototyping and reproducible upscaling of microfluidic building block production. The moulds consist of a bottom part with multiple standardized cavities and a top part that can have various inserts. The number of inserts ranges from one up to the number of cavities in the bottom part. Depending on the desired scale of production, one can choose the number of parts to be moulded. For medium-scale production, all cavities in the bottom can be exploited while in case of rapid prototyping one could use a single insert only. The method allows for rapid prototyping of thermoplastic microfluidic devices while remaining fully compatible with large-scale industrial fabrication.

Thanks to their design, individual building blocks can be used stand-alone using standard Luer- or mini-Luer connectors to connect to external lab equipment. However, combination of the microfluidic building blocks with a Fluidic Circuit Board (FCB) allows modular and versatile parallelization, multi-organ combination and sensor integration. Topics that are considered to be key hurdles in the large-scale adoption of organs-on-chips.

Because the building blocks and FCB are conform ISO22916:2022 the entire microfluidic platform is compatible with industrial commercialization and future organ-model integration.

References

[1] https://www.iso.org/standard/74157.html

Presentation: Oral

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Analysis of RNA content of anaerobic microbiota derived extracellular vesicles using organs on a chip

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The gut microbiota plays a crucial role in human health and is involved in a wide range of physiological processes. One of the ways that the microflora can influence these processes is through the production of extracellular vesicles (EVs) and their RNA content. Through the lumen, these EVs can enter the circulation, making them an important area of study in understanding the mechanisms of gut microbiota communication with host cells. However, current research methods are limited. One of the promising model systems for researching these processes is the organ chip (OC) platforms. To this end, we have developed a new method in anaerobic microflora research – a PDMS-free gut-on-chip (GoC) device.

We have also successfully optimized anaerobic microbiota isolation and co-cultivation within the GOC environment developed from stable cell lines, which was confirmed by metagenome sequencing data. Currently, we are analysing the EV RNA content of gastric cancer patient-derived microbiota, and how this content differs between EVs retained in the gut lumen and those that can pass through the gut-endothelial barrier. In conclusion, our research aims to provide a better understanding of the mechanisms and processes involved in gut microbiota communication via extracellular vesicles with host cells and their role in cancer progression and to develop new methods for studying these processes.

Funding: Project No.: 1.1.1.1/21/A/079

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Bioprinting effects on organoid proliferation, differentiation, and metabolism

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Combining 3D organoids with bioprinting provides a highly tunable microphysiological system, however understanding of the changes introduced by the printing process is lacking. In traditional 2D cell culture common assays used to characterize cells include vital dyes, metabolic assays, and morphological analysis. Often these characterizations are relatively straightforward as cells are grown in a single layer. The addition of a 3rd dimension adds challenges to these traditional characterizations. During the bioprinting process materials undergo shear stress, which could lead to various changes. Using colorectal patient derived organoids (PDOs) from normal and tumor tissue, we examined the effect of bioprinting on PDOs, specifically viability, proliferation and differentiation over time. Additionally, we examined specific cell type survival within the bioprinted PDOs using immunofluorescence (IF).

PDOs were harvested from basement membrane extract (BME) and directly printed intact, or digested to single cells prior to printing. Samples were encapsulated in hydrogels, then casted (controls) or printed with a drop-on-demand bioprinter. Viability was measured at multiple time points using vital dyes. Metabolism via ATP was measured using a luminescent assay. Organoid formation and morphology were imaged and tracked over time using brightfield and fluorescent microscopy. We compared printed and casted PDOs using vital dyes, IF, image analysis and ATP levels. Organoid formation, size and differentiation were tracked to determine recovery after the bioprinting process. Control PDOs were larger and more differentiated than bioprinted samples. ATP luminescence showed that bioprinted PDOs initially proliferate slower than the control but outpace the control by day 3. Taken together this data suggests that the shear stress during the printing process breaks up PDOs similar to the normal passaging process. This results in control PDOs exhibiting faster differentiation followed by death, while the bioprinted PDOs must first reform prior to differentiation.

Using tools adapted from traditional 2D cell culture methods we were able to characterize bioprinted and non-printed PDOs. The bioprinting of microphysiological systems is already a reality but it is vital to understand its impact at the molecular level.

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The characterization of a kidney cancer microphysiological system to investigate the impact of the SARS-CoV-2 virus spike protein on renal pathophysiology

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Kidney-on-a-chip models are becoming frequently employed to study morphology, disease development pathways, drug delivery, and renal toxicity. We have recently implemented a kidney cancer microphysiological system (MPS), based on the TissUse HUMIMIC Chip 2, that incorporates both healthy tubular epithelium and renal cell carcinoma cells, with the objective to mimic the immunogenic and angiogenic nature of renal carcinoma cells (RCC).

This platform allows us to conduct functional studies and investigate the influence of RCC in co-culture with healthy renal cells (RPTEC). Healthy-tumor cell interactions in MPS mimic the physiological environment of the kidney. Bespoke 3D-printed culture chambers were used to cast hollow gels that can be populated with non-tumor RPTEC-TERT1 cells to recreate a renal proximal tubule. Caki1 cells embedded in an agar/collagen gel were used to model RCC. In the HUMIMIC chip 2 recirculating flow connects the microenvironments of both renal tubules and RCC cells. Cells in MPS are polarized and maintain their connection with the surrounding tissue, forming a tight tubule. They receive and respond to extrinsic stimuli from the adjacent Caki1 cells that in MPS also develop into polarized spheroids, without direct cell-to-cell contact, and *vice versa*. In this model, the expression of immune factors (IL-6, IK-8) is downregulated in RPTEC-TERT1 while substantially upregulated in tumor cells. Tumor cells also preclude the expression of tight junctions (ZO1) and tumor necrosis factor (TNF) while upregulation of vascular endothelial growth factor (VEGF).

The SARS-CoV-2 spike protein (SP) is essential for attaching and recognizing cellular receptors and enables the coronavirus entry into host cells. Preliminary results show that SP seemingly does not affect non-tumor cells. On the other hand, the expression of immune factors is downregulated in tumor cells after SP exposure. These findings show the potential of using this novel kidney-cancer – MPS to investigate the immunomodulation of RCC.

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Application of an autologous human 3D vessel-on-a-chip cytokine release assay system to predict the safety of novel biologics

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Developing models that recapitulate the vascular endothelial/immune cell interaction *in vitro* is of great interest, particularly in relation to cytokine release syndrome (CRS): a severe and potentially life-threatening condition, where a sudden and rapid systemic release of pro-inflammatory cytokines is elicited in response to therapeutics. Following the failure to predict the CRS induced by TGN1412 in 2006, the need for improved predictive preclinical *in vitro* safety assessments has become a priority and a regulatory expectation to increase patient safety and reduce the costs associated with drug development programs. Current *in vitro* cytokine release assays (CRAs) using Peripheral Blood Mononuclear Cells (PBMCs) and Whole Blood (WB) utilise standard formats of antibody presentation (dry coat, wet coat or liquid phase). While these assays are biologically simple to run, they do not accurately reflect human *in vivo* physiology, and the limitations of toxicology models are well documented. In an effort to address this, we previously developed a fully autologous, human CRA using Blood Outgrowth Endothelial Cells (BOECs) derived from adult peripheral blood [1], co-cultured with PBMCs or WB from the same donor. This has become a routine assay to support various therapeutics in early stage drug development. We have recently expanded the assay into a 3D microfluidics system from MIMETAS, further recapitulating the physiological microenvironment of a blood vessel. Preliminary data demonstrated the ability of this model to successfully reflect the expected response to a range of clinically understood therapeutic antibodies, such as anti-CD3 (OKT3), anti-CD28 (TGN1412 analogue and ANC28), anti-CD52 (Campath) and Trastuzumab (Herceptin), without the need for the compound manipulation required in alternative assays formats. Here, further refinement and expansion demonstrates how this autologous microfluidic vascular model offers a sensitive, market-ready tool for the detection of CRS in preclinical investigations of candidate new drugs before the transition to first-in-human studies.

Reference

[1] Reed, D. M. et al. (2015). An autologous endothelial cell: Peripheral blood mononuclear cell assay that detects cytokine storm responses to biologics. *FASEB J 29*, 2595-2602. doi:10.1096/fj.14-268144

Presentation: Poster

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Inherently porous polycaprolactone substrates for *in vitro* 3D breast cancer cell culture for MPS applications

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Cancer is a becoming a huge social and economic burden on society, being the most significant barrier to life expectancy in the 21st century. One of the most significant difficulties to finding efficient therapies for specific cancers, such as breast cancer, is the efficiency and ease of drug development and testing. Micro-physiological systems (MPS) are under development to mimic the structural and biological complexity of human tissue, thus, becoming increasingly popular as an alternative to animal testing for pharmaceuticals [1]. However, the lack of a system for perfusion within current models remains a challenge.

High molecular weight polycaprolactone methacrylate (PCL-M) was used to fabricate polymerised high internal phase emulsions (polyHIPEs) [2] scaffolds to support 3D breast cancer (MDA-MB-231) cell culture within MPS systems. The effect of varying properties on polyHIPE porosity, interconnectivity, morphology, and mechanical properties was assessed via SEM and tensile testing. MDA-MB-231 cells were used to assess cell morphology, metabolic activity, and migration on PCL-M polyHIPE scaffolds. An *ex ovo* chick chorioallantoic membrane (CAM) assay was used to assess the biocompatibility of the polyHIPEs and potential for vascular invasion.

Via varying mixing speed and gelatin concentration within the internal phase the creation of interconnected micro and macro pores with varying mechanical properties was achieved, demonstrating the tunability of these polyHIPEs. *Ex ovo* CAM assays identified the scaffolds as bioinert, with biocompatible properties within a vascularised tissue. Furthermore, *in vitro* assessment of cell viability and proliferation showed promising potential for the use of PCL-M polyHIPEs to support breast cancer cell growth.

Photocurable high molecular weight PCL-M polyHIPE scaffolds show promise as a substrate for breast cancer cell culture with potential for vascularisation and active perfusion due to ease of tunability.

The EPSRC, centre for doctoral training in Advanced biomedical Materials for PhD studentship funding (EP/S022201/1)

References

[1] Knight, E., Murray, B., Carnachan, R. et al. (2011). Alvetex®: Polystyrene scaffold technology for routine three dimensional cell culture. *Methods Mol Biol 695*, 323-340. doi:10.1007/978-1-60761-984-0_20

[2] Aldemir Dikici, B., Claeyssens, F. (2020). Basic principles of emulsion templating and its use as an emerging manufacturing method of tissue engineering scaffolds. *Front Bioeng Biotechnol 8*, 875. doi:10.3389/fbioe.2020.00875/bibtex

Presentation: Poster

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A microphysiological human renal tubulointerstitium model as a testing platform for drug-inducing nephrotoxicity and dynamics of infectiosity

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Current experimental models have proven their limitation to understand biology of kidney diseases such as nephrotoxic drug-induced acute-kidney-injury (AKI) as well as viral nephropathies. Furthermore, murine or non-human primate models do not faithfully recapitulate the kidney microphysiology and these are almost irrelevant for investigating BKPyV infection affecting humans. Recent developments in bioengineering, microfluidics and human cellular biology provide the opportunity to develop innovative *in vitro* human kidney, mostly mimicking the proximal tubule.

We recently set up a human proximal tubule or tubule-on-chip (cToC) based on the Humimic chip, an off-the-shelf, two-circuit microfluidic chip, channel-seeded with telomerase-immortalized RPTECs or hTERT1-RPTECs. A continuous cell monolayer formed after four days under a finely regulated, pulsatile fluid flow which exerted a fluid shear stress (FSS) on the apical part of RPTECs.

We demonstrated cells could colonize the whole inner surface of the microfluidic channel and analyse cellular gene/protein expressions in that setting compared to a static hTERT1-RPTEC culture. Further, we showed RPTECs were capable of glucose and albumin reabsorption in the cToC configuration. Altogether, these preliminary results suggest cToC exhibits key phenotypical and functional key features of the native proximal tubule.

The ongoing tests demonstrate that hTERT1-RPTEC are permissive to different strains of BK polyomavirus. Allowing us to start investigating dynamic of infection in the perfusion condition.

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Polymorphonuclear neutrophils and monocytes are circulating vectors of transmission in the establishment of secondary infection foci by *Staphylococcus aureus* in a perfused model

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Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) causes severe tissue infections and sepsis. With a high mortality rate, infections continue being a major burden for global health. There is increasing evidence that *S. aureus* persists within host cells, thereby causing chronic and recurrent infections. Peripheral immune cells could act as a "Trojan horse" for the dissemination into peripheral organs [1].

In monoculture experiments, monocytes and neutrophils could be identified as potential vehicles. Therefore, various time points from the acute phase up to 24 h of bacterial persistence were analyzed. Initially, the bacteria were internalized equally by monocytes and neutrophils, showing a maximum of viable intracellular bacteria after 60 min. Monocytes could be characterized as a preferred bacterial persistence niche after 24 h. Further, the function of the *accessory gene regulator* (*agr*) operon controlling the main virulence factors of *S. aureus* was investigated using an *S. aureus agr* knock-out strain (*agr* -) [2]. 24 h post-infection, the number of *agr*- *S. aureus* was significantly higher compared to the wildtype strain in both cell types.

Thereupon, the potential of the infected peripheral immune cells as vectors of transmission was evaluated in a linear perfused model of the vasculature, in the presence and absence of tissue-resident macrophages. By analyzing the effluent and immunofluorescence staining, we could demonstrate that the transmission is strikingly dependent on the previous infection time of monocytes/neutrophils and tissue-resident macrophages represent a vital target for the establishment of secondary infection foci.

Studying infection routes by more complex MPS, such as gut and lung models, will gain more detailed insight in future on the complex pathogen-host interaction. These model systems will help in identifying new therapeutic targets for chronic and relapsing infections.

References

[1] Thwaites, G. E. and Gant, V. (2011). Are bloodstream leuko-

cytes Trojan Horses for the metastasis of Staphylococcus aureus? *Nat Rev Microbiol 9*, 215-222.
[2] Tuchscherr, L., Medina, E., Hussain, M. et al. (2011). Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol Med 3*, 129-141.

Presentation: Poster

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Smart Multi-Well Plate: Industrializable open technology platform for tubeless, autonomous OoC applications

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Introduction: Organs-on-Chip (OoC) have been a promise of microfluidics since their early days, leading to a widespread use in research. OoC adoption by industry conversely has so far been slow, resulting in a big gap between what is developed by elite pioneers and what can be offered to a broad audience. A way to bridge this gap is the consolidation of standards for design, manufacturing and qualification of OoC, making it easier for experts in different fields to combine their expertise and creating integrated systems with higher value than the sum of the parts. To this end, here we present the Smart Multi-Well Plate (SMWP), a device based on an open technology platform and the standardized 96-well plate format. Within this format, the SMWP integrates 16 OoC devices and piezoelectric micropumps, connected by a microfluidic network and controlled by integrated electronics.

Results and discussion: The SMWP allows for high modularity, with design and interfacing rules according to guidelines defined by experts from industry and academia [1]. With the SMWP, OoC devices, sensors and piezoelectric micropumps can be integrated using industrial workflows exploiting automated pick-and-placing and wire-bonding machines. The modules are fluidically connected by means of a polymeric fluidic circuit board and electrically connected by reusable and disposable printed circuits boards. These take care of both control of micropumps and sensors and transfer of power and data to the outside world. Fluidic and electric performance of the plate, including piezoelectric micropumps from Fraunhofer EMFT, were tested in combination with barrier model OoC devices from BEOnChip and Bi/ond and multi electrode array chips from MultiChannel Systems. Preliminary results of the biological validation of the three embodiments of the SMWP will also be presented.

Conclusions: An autonomous standardized OoC platform based on an open technology was realized by leveraging industrially upscalable processes and integrating OoC devices.

This work is part of the Moore4Medical project funded by the ECSEL Joint Undertaking under the grant number H2020-EXCEL-2019-IA-876190.

Reference

[1] van Heeren, H. et al. (2017). MicroFluidic Manufacturing White paper. doi:10.13140/RG.2.1.3318.9364

Presentation: Oral

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Investigating the crosstalk between cardiomyocytes, fibroblasts, endothelial cells and resident macrophage within vascularized cardiac organ-on-a-chip platforms

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The advances in human stem cell technology and biofabrication techniques have generated a synergetic influence to accelerate research in the field of heart-on-a-chip. Functional cardiac tissues with microvascular plexus remain the challenge due to the highly condensed and organized tissue architecture and the complex multi-cell interactions. In the heart, fibroblasts, endothelial cells, and circulating and resident macrophages (Macs) play an important role in matrix deposition, vascularization, and paracrine signaling. Cardiac resident macrophages, which originate from the yolk sac, were shown to facilitate angiogenesis [1], cardiomyocytes (CMs) proliferation, improve electrical conduction [2], and promote scarless repair post-MI [3]. Thus, the incorporation of Macs along with other cells is critical to achieving an adult-like, functional, vascularized engineered cardiac tissue.

Here, we first evaluated vessel network formation within a cardiac tissue using a fibrin plug method and identified that when culturing ECs with CMs, vessel networks formed but degraded over time, whereas the control cultures, without CMs, remained stable for weeks. Moreover, pro-inflammatory and endothelial activation cytokines secretion were increased in the presence of CMs. When adding Macs to the culture, vessel stabilization occurred along with a decrease in pro-inflammatory and endothelial activation cytokines secretion.

We then cultured Biowire tissues [4] to evaluate the cardiac function of the vascularized cardiac tissues with and without Macs for up to 4 weeks, with a weekly assessment of contractile dynamics over a range of frequencies, electrical excitability (excitation threshold, maximum capture rate) and Ca^{2+} transient dynamics. A higher force of contraction and MCR were observed in the group with Macs. Last, we evaluated vascular function within the cardiac tissue using the iflow plate. The vascularized tissues with Macs remained perfusable after 4 weeks of culture, whereas tissue with no Macs clogged. In summary, we demonstrated the importance of adding Macs to a micro-vascularized heart-on-a-chip platform that can serve as a versatile tool to the research community for disease modeling and drug testing with higher clinical relevance.

References
[1] Epelman, S. et al. (2015). *Nat Rev Immunol.*
[2] Hulsmans, M. et al. (2017). *Cell.*
[3] Dick, S. A. et al. (2019). *Nat Immunol.*
[4] Zhao, Y. et al. (2019a). *Cell.*

Presentation: Oral

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Stem cell-derived gut-on-chip technology to elucidate human norovirus infections

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Introduction and project outline: Gastrointestinal inflammations caused by pathogens are severe infections affecting millions of people worldwide in an age-independent manner. Infections with the Human Norovirus (HuNoV) are causative for acute gastroenteritis (AGE) and are associated with nearly 20% of all acute diarrheal cases globally [1]. However, to date, no effective treatment or vaccine for HuNoV infection is available. Animal models are not capable of mimicking HuNoV infections *in vivo*, and there is a considerable lack of appropriate *in vitro* models reflecting the required complexity of the human intestine including its mucosal immune system. Recent studies demonstrated the successful infection and replication of HuNoV using intestinal epithelial cells derived from adult stem cells (ASCs) and identified bile acids as a strain-specific requirement for effective HuNoV infection and its replication [2,3]. We thus built up a biobank of ASC-derived human intestinal organoids and stool sample filtrates for the generation of patient-specific gut-on-chip models to investigate HuNoV infection and to explore the potential of these organ-on-chip models to study correlates of protection for HuNoV in individuals of FUT2 secretors and non-secretors.

Experimental procedure: To establish a HuNoV infection model, we use patient-derived intestinal epithelial cells and human stool samples of infected patients co-cultured in a gut-on-chip model and analyse viral replication. Infection-related tissue alterations will be investigated in the presence of sterile stool sample filtrates and bile of healthy donors and infected patients.

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 101007799 (www.imi.europa.eu). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

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References
[1] Lucero, Y., Matson, D. O. et al. (2021). *Viruses 13*, 2399
[2] Constantini, V., Morantz, E. K. et al. (2018). *Emerg Infect Dis 28*, 1453-1464.
[3] Murakami, K., Tenge, V. R. et al. (2020). *PNAS 117*, 1700-1710.

Presentation: Poster

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Process for bonding separate substrates by gelatin coating: Applications in microfluidics

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New and reversibly bonded chip are urgently needed to enable industrial applications and to support the formation of microphysiological systems [1]. A smart, tunable and room-temperature bonding process via gelatin dehydration was developed for the fabrication of microfluidic chips patterned with microchannels [2]. Recently, we improved the protocol of dehydration reducing drastically the time to few hours. The tough and reversible bonding can be obtained by coating a thin layer of gelatin onto the different

substrates and, consequently through the contacting and clamping, a dehydration process forms a thin membrane between the substrates. The performance of the sealing was tested by burst pressure tests with regards to the most commonly used combination of materials in microfluidic cell culture. For most materials, the bonding resisted until ~6 bar and remained functional until 15 days in a cell culture incubator. This method is applicable in rapid prototyping of microfluidic cell culture, microelectronic components and in delicate environments such as biomedical devices inaccessible for high temperature treatments and chemicals cross-linking.

References

[1] Teixeira Carvalho, D. J., Moroni, L. and Giselbrecht, S. (2023). Clamping strategies for organ-on-a-chip devices. *Nat Rev Mater*.
[2] Pitingolo et al. (2019). *Adv Eng Mater*, 1900145

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A new barrier-on-chip system for straightforward workflow integration

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MPS replicate the smallest functional units of organs *in vitro*, to better model human physiology. Recapitulating different organ barriers, with their sophisticated and dynamic microenvironment and cellular complexity, using a single system remains a challenging task. Aside the biology and engineering, there are challenges in how the MPS can best be integrated into existing laboratory workflows including handling and readouts.

The AXBarrier-on-Chip system is an easy-to-handle platform and compatible with standard workflows. The system allows recreating different organ barriers like lung, gut, bladder, skin, and many more. This is done by resembling key aspects of the organ microenvironment like dynamic motion, ultra-thin, porous, elastic, and soft cell culture membrane, and fluid flow. The consumable of the AX-Barrier-on-Chip system, called AX12, allows easy cell seeding on either side of the integrated membrane, and readout possibilities. Based on the SBS footprint the AX12 is designed for simple integration into existing workflows. In addition, the chip design is tailored for accurate *in vitro* to *in vivo* translation using multiple readouts: Use automated high-resolution and real-time imaging to visualize dynamic biological processes or e.g. target expression. Apply Trans barrier Electrical Resistance (TER) and permeability assays to assess organ barrier disruption e.g. due to toxic compounds, or viral/bacterial infection. Use ELISA/proteomics, on the up-concentrated supernatant, to assess secreted biomarkers or to investigate immune-mediated cytokine storm. Perform Single-cell transcriptomics to evaluate the *in vitro* population and correlate it to *in vivo*.

Supported by the unique design, format, and different readout possibilities, the AXBarrier-on-Chip system facilitates a straightforward workflow integration. It further enables a fast MPS translation of existing protocols to the AX12. The generated data while assessing the safety and efficacy of new drug candidates support the potential of this new barrier-on-chip system to better assist decision-making, routine use in the preclinical phase of the drug development, and its valuable alternative to animal experiments.

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Direct evaluation of substrate's effects on intestinal epithelium cell cultures through a novel MPS for unbiased comparative studies

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The intestinal epithelial barrier plays a pivotal role in the molecular absorption process. Although complex intestinal *in vitro* models are needed to carry out reliable pharmacological investigations, cell cultures are mainly performed using standard culture systems in which cells are cultured on membranes in static conditions, thus failing in replicating the micro-physiological environment [1].

We developed a modular bicompartmental versatile culture system, named True Tissue on Platform (TTOP), characterized by a cartridge-based design which allows to host tunable culture substrates, such as microporous membranes or scaffolds. After the biological validation with human colorectal adenocarcinoma epithelial cell line (Caco-2), we focused specifically on the substrate's features. We compared different coatings and complex substrates, such as gelatin-based scaffolds and silk fibroin-based scaffolds, with respect to standard microporous membranes. In order to assess the barrier functionality, the Trans Epithelial Electric Resistance (TEER) was measured every two days. At the same time, TEER values were compared with fluorescence microscopy images obtained from the live staining of the samples (Hoechst). Controls were fixed and stained (DAPI, F-actin, Villin, ZO-1) at each time point. At the end of the culture all samples were fixed, stained and imaged with fluorescence microscopy.

The results point out that gelatin-based substrates promote proliferation and better support colonization, and suggest that the for-

mation of three-dimensional constructs is enhanced accordingly. Caco-2 cultured on bare polycarbonate membranes reached confluence after 14 days, TEER values kept increasing until day 25, according to the state of differentiation seen in controls. On gelatin-based substrates confluency was achieved after only 7 days. Moreover, after 10 days, TEER of gelatin-based samples was significantly higher ($p \leq 0.001$) with respect to Caco-2 cultured on bare polycarbonate membranes.

Contrarily to previous studies which adopted different devices to evaluate diverse substrates, TTOP enables to host different substrates without altering other environmental conditions, thus directly considering the influence of the culture surface. The possibility to compare various substrates in an unbiased manner, will enable the tuning of the extracellular environment that, coupled with other features, like oxygen gradient and samples perfusion, will enable the development of a predictive intestinal model.

Reference

[1] Costa, J. and Ahluwalia, A. (2019). *Front Bioeng Biotechnol*. doi:10.3389/fbioe.2019.00144

Presentation: Poster

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Stimulating 3D skeletal muscle microtissues in a novel perfusable microphysiological system with integrated electrodes

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Three-dimensional engineered skeletal microtissues can provide great insight into disease modeling, drug development, and toxicity studies. However, developing *in vitro* models for skeletal muscle tissues has been particularly challenging due to the high metabolic demand, complex cell organization, and electromechanical functioning of native tissue. Current models often require the manual handling of external electrodes to stimulate muscle tissue, limiting throughput and increasing the chances of cell culture contamination. While multielectrode arrays solve this issue, they often lack perfusion for a steady influx of nutrients or pharmacological treatments. To address these limitations, we present here a novel microphysiological perfusion system with integrated microelectrode arrays to stimulate engineered 3D skeletal muscle tissue contraction *in situ*.

As proof of concept, we integrated sensors into our perfusable microchip containing two bioinert pillars. These pillars serve as anchoring sites, allowing for the 3D uniaxial alignment of skeletal myocytes as they form muscle bundles and mature into 3D microtissues. We show that the integrated electrodes can electrically stimulate the muscle microtissues *in situ*, by measuring the rate and magnitude of muscle contractions using microscopy-based image analysis. To ensure the relevance of our model, the differentiation and maturation into multinucleated contractile myotubes, with or without electrical stimulation, was characterized using fluorescent microscopy. In summary, we present a microphysiological system capable of simultaneous electrical stimulation and perfusion of engineered 3D muscle microtissues. This paves the way for more physiologically relevant biomimetic models in drug development and disease modeling.

Presentation: Poster

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Predicting renal drug clearance using mechanistic modeling based on drug secretion in a kidney microphysiological model

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During early development, a robust prediction of pharmacokinetic parameters is crucial to ensure the efficacy and safety of new drugs. Estimating renal clearance is particularly important given that a majority of small-molecule drugs are excreted via the kidneys. Conventional models are limited in their capability to recapitulate the intricate machinery of the renal proximal tubules and are notorious for the loss of drug transporters, which precludes accurate predictions of drug secretion. Advances in microphysiological systems (MPS) have enabled the development of more representative renal tubule models that can retain the native transport activity of the kidneys. In the present study, a kidney-MPS was used to effectively recreate drug secretion and coupled to mechanistic modeling to predict renal drug clearance. Renal tubules comprising of human proximal tubule epithelial cells (RPTECs) grown in the kidney-MPS recreated a tight, impermeable epithelium with defined basolateral and apical polarization. The kidney-MPS actively secreted the organic cation drug metformin and the organic anion drug cidofovir, in contrast with RPTECs grown in conventional trans-well cultures. The permeability of metformin and cidofovir was estimated using a semi-mechanistic model of drug distribution in the kidney-MPS. Subsequently, these parameters

were employed to predict renal secretory clearance using *in vitro* to *in vivo* extrapolation. The renal clearance predictions for metformin and cidofovir were within 3.3-and 1.3-fold, respectively, of clinically reported values. This approach shows that kidney-MPS models coupled with pharmacokinetic modeling are effective tools to evaluate drug clearance. By providing human-relevant predictions, kidney-MPS-derived parameters can complement pharmacokinetic studies and contribute to reducing the use of pre-clinical animal species during pre-clinical drug development.

Presentation: Oral

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The alveolus in the spotlight: How to translate lung *in vitro* studies into clinically relevant outcomes

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Despite the high importance of the distal lung as both ultimate barrier and vital function support, *in vitro* model development has been rather slow and not highly consistent until the past 10 years. COVID19 has fueled a surge of interest in relevant alveolar models, which has converged with the window of opportunity offered by the birth of MPS. Together with the novel regulatory landscape supporting more humane testing as opposed to animal modeling, new opportunities are arising in the respiratory research area requiring robust and validated *in vitro* models of the alveoli.

In this work, we describe the AXLung-on-chip system and assess its preclinical relevance in a case-to-case basis. Different biomodels enable the approach of biological contexts of interest permitting PK evaluation (molecule transport and uptake); hit and target identification (protein secretion, immunofluorescence, transcriptomics); structural and cellular microtissue rearrangement (immunofluorescence, histology, scRNAseq) and molecule-induced toxicity or vascular leak.

By using immune competent systems including biologically meaningful cell-cell crosstalk, we have evaluated immunotherapeutic drugs (FolR1-TCB; Proleukin®) resulting in valuable inputs that confirmed safety concerns (increased inflammation, immune cell recruitment, barrier disruption). Further, alveolar primary cell-based models, not only recreate utmost alveolar characteristics but also, enable the assessment of individual patient variations for drug targeted therapy and personalized medicine. Finally, both primary cell-based and immortalized cell line models (Sengupta et al., 2022) have proven successful in the development of efficacy models of chronic diseases with limited therapeutic option such as ARDS or pulmonary fibrosis. Thus, offering an innovative and cost-effective way to evaluate therapeutic intervention, which would be otherwise challenging and costly considering the complex etiology of these diseases.

Altogether, our results show that the AXLung-on-chip technology replicates accurately the alveolar microenvironment and sustains cellular models that capture clinically relevant features. Its application as a target identification and drug testing tool is supported by several regulatory submissions, underlining its translational potential and use to assist decision-making within the drug development pipeline while minimizing animal modeling.

Presentation: Poster

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A novel gut-on-chip model recreating physiological 3D peristalsis

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The highly mechanically active human gut represents a key element in the uptake of nutrients and orally administered drugs. Replicating biomechanical stimuli is essential to preserve *in vivo* phenotypes and predict physiological cell responses. In the gut context, recapitulating 3D peristalsis is therefore of high relevance to simulate *in vivo* conditions. However, its complex nature and dynamic variations during the day, has made it difficult to recapitulate *in vitro*.

In this study, we have developed a new gut-on-chip model using the AXBarrier-on-chip system. The gut epithelial barrier was recreated on the AX12's ultrathin porous membrane using the validated gut cell lines Caco-2 and HT29 that represent enterocyte-like and goblet-like cells respectively. Cell models were used as mono- and co-cultures on-chip and on traditional Transwell® inserts as a reference. To reproduce *in vivo*-like peristalsis, 3D stretch was applied using the AXBarrier-on-chip system considering physiological variations during the daytime (active) and nighttime (resting).

Both mono- and co-culture gut models on-chip and Transwell® were characterized by tight barrier formation (Trans barrier Electrical Resistance, TER). 3D peristaltic stimulation on-chip resulted in TER and permeability values closer to *in vivo* parameters, which is essential to replicate relevant molecule transport across the barrier for PK modeling. Additionally, on-chip conditions fostered cell differentiation and localized F-actin filaments indicative of cell polarization and increased gene expression of typical gut markers (ANPEP, LYZ, MUC2) when compared to the Transwell® platform. Finally, we evaluated our model for efficacy ap-

plications simulating the proinflammatory context associated to severe gut disorders such as inflammatory bowel disease. For that, we treated our model with a proinflammatory cocktail and evaluated the impact in barrier function and secretion of proinflammatory cytokines. Our results showed that 3D peristaltic stretched cells demonstrated a higher sensitivity resulting in increased inflammation and barrier weakening compared to the static model.

In summary, we successfully developed a dynamic gut-on-chip model reproducing physiological 3D peristalsis with *in vivo*-like barrier parameters and increased the sensitivity to proinflammatory triggers. This model represents an important step forward for more predictive toxicity evaluation, disease modeling, PK studies and drug safety and efficacy assessment.

Presentation: Oral

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Developing guidelines for microfluidic-based systems: A window into the future standardization of microphysiological systems

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An exponentially increasing number of microfluidic-based devices have been submitted for evaluation to the US Food and Drug Administration [1]. However, to help accelerate not only the evaluation process of these biomedical devices, but to improve manufacturability and interchangeability, and to foster the transition from laboratory to commercialization, the development of guidelines and standards is needed. Currently, the Microfluidics Association (MFA) is working towards the development of guidelines and standards for engineering aspects of microfluidics such as flow control, interconnections and component integration, to name a few. The MFA has already developed and published ISO 22916:2022 in which the requirements for the sound integration of microfluidic components within systems were presented [2]. This ISO standard should better guide the design of new microfluidic devices. Some of these engineering features and their future standards will directly impact microphysiological systems (MPS). For example, work towards new approaches to measure accurately minute flow changes within a microchannel network will inform the development of standard methods to accurately determine the flow rate within organ models. These approaches are expected to greatly improve confidence in these platforms. Furthermore, fluidic shear stress measurements have been viewed as one of the two areas considered as critical aspects in need of standardization by the MPS community [3]. Thus, having standard methods to measure flow accurately will provide a reliable approach to attain shear stress values within MPS. Here, we present important advancements the MFA has made in several areas related to the standards of microfluidic systems. Specifically, work is currently being done towards the development of a roadmap that will inform the development of standards for: 1) the characterization of flow resistivity, 2) protocols for leakage testing, 3) parameter datasheets for pumps and other microfluidic components, 4) interfacing of optics and electronic sensors, and 5) the needs of MPS, with the help of CEN-CENELEC Organ on a Chip working group.

References

[1] Reyes et al. (2021). *Lab Chip 21*, 9-21.
[2] Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification, ISO 22916:2022, Switzerland, January 2022
[3] Piergiovanni et al. (2021). *Lab Chip 21*, 2857-2868.

Presentation: Oral

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Human mesenchymal, liver, and endothelial cells self-organization in spheroids in an acoustofluidic microphysiological system depends on cell type-specific mechanical properties

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Improving the sensitivity and accuracy of predictive assays is a pressing challenge for the drug discovery pipeline. In order to detect drug efficiency and toxicity in preclinical phases, pharmaceutical stakeholders recommend combining different predictive models, including 3D *in vitro* organ models such as microphysiological systems (MPS) [1].

We developed a microphysiological system that allows the synergetic combination of ultrasonic manipulation and cell culture. Ultrasounds can be used to safely control the position of cells inside a microfluidic chip via contactless handling [2]. In our polydimethylsiloxane (PDMS) chip, the Acoustic Radiation Force (ARF) aggregates the cells away from the walls [3] and can maintain them in acoustic levitation during several days. Our acoustic traps enable rapid cell-cell contacts within a few seconds. In a single chip more than 20 compact cell sheets can be gently formed in acoustic levitation. In these conditions, self-reorganization of cell sheets into 3D structures were monitored for 3 days. Resulting spheroids were found viable, cohesive, and reproducible in size and shape. Our system thus represents a powerful technology for the production of human cells spheroids.

By using two types of liver cells (HepaRG cells and cryopreserved human primary hepatocytes), Human Umbilical Vein Endothelial Cells (HUVECs), and Mesenchymal Stromal Cells (MSCs), we showed that different self-organization dynamics occurred, based on each cell type physiological and mechanical properties, such as contractility.

Inside our microfluidic chip, spheroids can be treated with various drugs, and later retrieved easily by turning the ultrasounds off. Such MPS could thus be of interest for rapid and efficient 3D cell cultures, cell mechanical studies, and robust and reproducible pharmaceutical assays.

References

[1] Weaver, R. J. et al. (2020). Managing the challenge of drug-induced liver injury : A roadmap for the development and deployment of preclinical predictive models. *Nat Rev Drug Discov 19*. doi:10.1038/s41573-019-0048-x

[2] Wiklund, M. (2012). Acoustofluidics 12 : Biocompatibility and cell viability in microfluidic acoustic resonators. *Lab Chip 12*, 2018-2028. doi:10.1039/C2LC40201G

[3] Jeger-Madiot, N. et al. (2021). Self-organization and culture of mesenchymal stem cell spheroids in acoustic levitation. *Sci Rep 11*. doi:10.1038/s41598-021-87459-6

Presentation: Poster

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Vascularized 3D airway-on-a-chip with air and media perfusion to study COVID-19 and other respiratory diseases

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In recent years, there has been a burgeoning interest in developing multi-cell type *in vitro* models that mimic the structure and function of human airways to study respiratory diseases such as COVID-19. The use of dynamic 3D organ-on-chips presents a promising approach as they provide a more physiologically relevant model for the study of virus-host interactions and drug efficacy.

We developed a multi-chamber chip made of a 3D-printed transparent photopolymer for the culture of hydrogels mimicking the airway mucosa and submucosa. Inside each of the chip chambers, a bioink composed of fibrinogen and loaded with primary human airway fibroblasts was deposited using a drop-on-demand bioprinter. The fibrinogen polymerizes in the presence of thrombin to form a solid hydrogel around a plastic molding line that can be removed to form a hollow channel. This channel was seeded with human umbilical cord endothelial cells (HUVECs). The endothelialized vessel was then connected to cell media perfusion to supply the model with nutrients and oxygen. Human airway epithelial cells were seeded on top of the fibrin hydrogels after printing and the models were cultured in submerged conditions for a week. When the epithelium was fully confluent, the media was removed and the top channel was connected to an air pump mimicking the human inhalation and exhalation, exposing the epithelial cells to air-liquid interface and wall shear stress. The chip's air compartment was designed based on computational fluid-flow simulations to assure a homogeneous laminar flow free of dead zones. The post-printing viability of the fibroblasts was evaluated by live/dead staining using calcein AM and propidium iodide, and imaging with two-photon laser scanning microscopy, confirming a cell survival rate of around 85%.

In conclusion, we present an airway-on-a-chip model composed of a 3D bioprinted fibrin gel nourished through an internal perfusable vessel and connected to air perfusion for the stimulation of

the mucosal epithelium. This chip can be used to study the effect of respiratory viruses such as SARS-CoV-2 on the airway epithelium and its interaction with the drugs present in the blood, providing clinically translatable data.

Presentation: Poster

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Microvascularized neurovascular unit (NVU) model using human induced pluripotent stem cells (hiPSC) and laser cavitation molding

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Treating pathological conditions in the human brain requires a complete understanding of brain physiology. Dedicated 3D tissue models must reproduce the tight control of matter exchange regulated by the brain microvasculature. We have recently advanced in laser micromachining of soft materials allowing for the generation of arbitrarily-shaped microvascular networks with minimal damage to the cells embedded in the hydrogel [1]. The dimension of the vessels and the biocompatibility of this approach are ideal for modeling brain tissue, but tissue-specific cells are necessary to recapitulate the neurovascular unit (NVU) complexity.

To address this limitation, we used vascular cells (pericytes and endothelial cells) [2] and cerebral organoids [3], both derived from human induced pluripotent stem cells (hiPSCs). Cerebral organoids were encapsulated in type-I collagen-based hydrogels. These organoid-laden hydrogels were exposed to near-infrared pulsed laser radiation (1040 nm, < 400 fs pulse length) at a high pulse frequency (0.1-1 MHz) to define the outlines of a vascular interface with the organoids. The formation of cavitation bubbles rearranged the fibrillar structure of the hydrogel, rendering continuous and stable microchannels (20-60 µm) in which brain endothelial cells were later seeded.

In summary, optimized femtosecond laser technologies allow for cavitation-based patterning of microchannels in hydrogels directly adjacent to extremely sensitive cellular assemblies such as organoids. The combination of this technique with hiPSCs enables the generation of more physiologically relevant human tissue models.

References

[1] Enrico, A. et al. (2022). 3D microvascularized tissue models by laser-based cavitation molding of collagen. *Adv Mater 34*. doi:10.1002/adma.202109823

[2] Pars, S. et al. (2021). Generation of functional vascular endothelial cells and pericytes from keratinocyte derived human induced pluripotent stem cells. *Cells 10*, 74. doi:10.3390/cells10010074

[3] Lancaster, M. et al. (2014). Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc 9*, 2329-2340. doi:10.1038/nprot.2014.158

Presentation: Oral

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In silico replication of hypoxia dynamics and readouts of an ischemia/reperfusion MPS for system identification and pharmacological investigations

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Introduction: Ischemic heart disease (IHD) stems from the mismatch in oxygen and nutrients supply and demand in the heart. Disturbed cardiac energetics and the intertwined mechanisms of ischemia/reperfusion (I/R) necessitate studying the phenomenon with cell models incorporating interorganellar interactions and elector-mechano-energetic coupling. The interest in experimenting I/R using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) grows rapidly due to the novel capabilities that organ-on-chip approaches offer in condition control and measurement modalities.

Method: Facilitated by the emerging *in vitro* hiPSC-CM I/R data [1,2], we developed a hiPSC-CM computational model including a metabolite-sensitive SERCA pump and a contractile element (CE) to investigate I/R and the effect and action mechanism of a Ca^{2+} sensitizer drug, Levosimendan (Levo). We also introduced a novel Oxygen dynamics formulation to fit ischemia/reperfusion (I/R) pathophysiology and validated the model using hiPSC-CM and animal *in vitro* data.

Results and discussion: Contribution of SERCA to the diastolic and contractile relaxation dysfunction in I/R was predicted similar to its mechanism in a sepsis-induced heart failure condition. Of note, Levo simulations revealed that the counteractive effect of the drug on the contractile relaxation dysfunction is due to the specific Ca^{2+} sensitizing mechanism of Levo through Ca^{2+} bound troponin

C and the Ca^{2+} flux to the myofilament rather than SERCA phosphorylation blockade.

Conclusions: This work introduces a novel I/R computational framework for the cardiac electro-mechano-energetic coupling in hiPSC-CMs, showing its deep-phenotyping and pharmacological potential. The model can serve as a platform to test I/R treatment ideas according to the temporal evolution of metabolites and molecular mechanisms in cardiomyocytes.

References

[1] Gaballah, M. et al. (2022). Cardiac ischemia on-a-chip: Antiarrhythmic effect of levosimendan on ischemic human-induced pluripotent stem cell-derived cardiomyocytes. *Cells 11*, 1045. doi:10.3390/cells11061045

[2] Häkli, M. et al. (2022). Electrophysiological changes of human-induced pluripotent stem cell-derived cardiomyocytes during acute hypoxia and reoxygenation. *Stem Cells Int 2022*, 1-15. doi:10.1155/2022/9438281

Presentation: Poster

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From materials to manufacturing: A journey towards microfluidics production standardization

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To better mimic the *in vivo* environment for organoid models and especially 3D-cultures, chips and devices become more and more complex, using various mechanisms for fluidic control. With the aim of improving and easing organ-on-chip development and studies, the microfluidic industry is developing some consensus-based measurement and guidelines, in particular with ISO standards (ISO 22916:2022). To improve device availability, development speed, and encourage large-scale adoption, generic protocols and material characterization are essential and begin to be used [1].

Polymers are mainly used for prototyping these devices. If PDMS is widely used as first material choice for proof of concept at the laboratory scale, it is widely replaced by other polymers and/or silicon or glass when it comes to scale up to commercial production. One of the most promising materials is Cyclic Olefin Copolymer (COC), with several advantages: nonporous, biocompatible, mainly inelastic, relatively cheap, and easy to manufacture. Moreover, its optical and electrical properties allow integration of sensors for imaging, conductivity measurements, impedancemetry [2]. However, it remains critical to standardized testing and calibrations methods [3].

In this work, we will present and compare two major ways to prototype microfluidic devices from COC for cell cultures and beyond: micro milling and hot embossing. These two techniques are both presenting pros and cons, whether in terms of time consuming, reproducibility, accuracy, simplicity, quality, and sensitivity. If micro milling is well adapted to develop low-volume prototypes, and iterate their designs, hot embossing allows producing larger volumes, in a reproducible way, without compromising the spatial resolution. Moreover, it allows better supported test protocols such as leakage tests.

Both techniques can also be implemented for the heterogeneous integration of sensors and different functions for scalability and automation. To support the microfluidics community efforts in product development and commercialization, this comparison gives a quantitative metrics to provide quality insurance before, during and after the manufacturing process.

References

[1] Parent, C. et al. (2022). *Lab Chip*.

[2] Roy, M. et al. (2014). *Sens Actuators B Chem*.

[3] Silveiro, V. et al. (2022). *Front Bioeng Biotechnol*.

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Cell-friendly 3D oxygen imaging for microphysiological systems

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Over the last few years, biological *in-vitro* disease modelling and preclinical drug testing have started to move from 2D towards more complex 3D systems, comprising various cell types in a physiological microenvironment. This trend necessitates a transformation of various measurement and imaging technologies to 3D as well. Indeed, there is a growing need to image both cellular structures and locally changing chemical key parameters, such as oxygen partial pressure (pO_2) or pH, in 3D. While both the ratiometric and luminescence lifetime microscopy (FLIM)-based 2D imaging of pO_2 and pH are relatively widely applied in the biomedical field today, the luminescence imaging in 3D generates new challenges, especially in long-term monitoring of cell cultures. Confocal microscopies in 3D result in a large cumulative light exposure, which can generate unwanted cell responses, let alone damages through phototoxic effects. For long-term luminescence-based imaging in 3D, light-sheet microscopies provide attractive alternatives for confocal technologies, as they can reduce the phototoxic effects even by orders of magnitude [1]. Here we introduce our 3D luminescence microscopy platform, combining two sophisticated luminescence

microscopy modalities: a light-sheet illumination scheme called selective plane illumination microscopy (SPIM) and fluorescence lifetime imaging microscopy (FLIM). The combination enables a fast and cell-friendly 3D sample scanning and a versatile use of chemical indicator particles with minimal light exposure. We report on the design, optomechanical realization and on the first results on 3D oxygen imaging. The results show that the platform has a great application potential especially with cell models, where hypoxic or strongly varying oxygen environments are of interest, including ischemic heart models, stroke models and cancer models, to name a few.

Reference

[1] Girkin, J. M. and Carvalho, M. T. (2018). The light-sheet microscopy revolution. *J Opt 20*, 053002.

Presentation: Poster

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A microfluidic model of human vascularized breast cancer metastasis to bone for the study of immune-cancer cell interactions

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The formation of organ-specific metastases of breast cancer to bone is driven by specific interactions between the tissue microenvironment and cancer cells (CCs). However, it is still unclear the role that blood circulating immune cells, including neutrophils, play during bone colonization. Indeed, previous studies have shown that neutrophils might acquire either a pro-tumoral or anti-tumoral phenotype [1]. In this project, we then aimed at analyzing the migration of neutrophils when exposed to breast CCs colonizing the bone and their role in the growth of micrometastases from breast cancer.

Based on our previous bone metastasis models [2,3], here we designed a microfluidic system that allows to independently biofabricate human vascularized breast cancer metastatic seeds within a bone-mimicking microenvironment embedding osteo-differentiated mesenchymal stromal cells and endothelial cells (ECs). Microvascular networks self-assembled and connected the bone-mimicking microenvironment with the breast cancer metastatic seed. Compared to controls without CCs, metastatic seeds altered the architecture of microvascular networks resulting in a lower number of junctions (5.7 ± 1.2 vs 18.8 ± 4.5) and shorter total network length (10.5 ± 1.0 vs. 13.4 ± 0.8 [mm]). In addition, vascular permeability was significantly higher in presence of CCs ($3.8 \pm 6.8 \times 10^{-5}$ vs. $5.3 \pm 4.4 \times 10^{-6}$ [cm/s]). Following maturation of the metastatic seeds, neutrophils were injected into microvascular networks. Neutrophil extravasation was significantly higher when CCs were present (27.9 ± 13.7 vs. 14.7 ± 12.4 [%]). Importantly, the percentage of dying CCs was higher in presence of neutrophils, as confirmed by confocal imaging and flow cytometry on isolated cells.

The biofabricated metastatic niche is a powerful model to analyze the biological mechanisms underlying the interaction between circulating neutrophils or other immune cells and organ-specific micrometastases. Moreover, this model represents an effective tool to test novel drug combinations targeting the bone metastatic microenvironment.

References

[1] Janssen et al. (2017). *J Immunother Cancer 5*, 1-14.
[2] Bersini et al. (2014). *Biomaterials 35*, 2454-2461.
[3] Jeon et al. (2015). *PNAS 112*, 214-219.

Presentation: Poster

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Development of an opto-microfluidic assay to probe signaling and function in glomeruli-on-chip

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The identification of signaling pathways in physiological environments may be essential to understand and possibly control complex physio-pathological processes. This is notably true for inflammation-related conditions such as Rapidly Progressive GlomeruloNephritis (RPGN). This pathology, for which ROS signaling is assumed to play a central role [1-2], causes terminal kidney failure. Quantitatively probing the cell organization and behavior at the functional and molecular scales *in situ* and notably identifying the loci, the timing and the quantity of such oxidative molecules could provide a new readout of RPGN pathological transition. We thus developed an opto-microfluidic biomimetic assay, combining a thorough glomerulus reconstitution on-chip to decipher complex migration processes and advanced quantitative imaging methods using lanthanide-based luminescent nanoparticles to achieve fast ROS detection [3] in cell microsystems.

We build these microsystems directly on an optically accessible surface, allowing monitoring of the cells from the individ-

ual molecule to the tissue response, with virtually any high numerical aperture microscope. We demonstrated the feasibility to co-culture 3 cell type/layer system mimicking the glomerular organization. Podocytes, Glomerular ENdothelial Cells (GENCs), and Parietal Epithelial Cells (PECs) expressed specific markers after 2 weeks of differentiation-on-chip. Besides, a functional assay showed filtration properties similar to what's expected of an actual glomerulus.

Our microfluidic biomimetic framework tends to bridge the gap between *in-vitro* and *in-vivo* studies by bringing a relevant complexity while answering a biological question at the "meso-scale" of a tissue construct. Our device also enables future combinations of techniques with molecular measurements such as super-resolution imaging-on-chip or single-molecule tracking.

References

[1] Bollée et al. (2011). *Nat Med 17*, 1242
[2] Lazareth et al. (2019). *Nat Comm 10*, 3303.
[3] Casanova et al. (2009). *Nat Nanotech 4*, 581.

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Key recommendations from GIVIMP for test system suppliers

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The Organization for Economic Cooperation and Development (OECD) guidance document on Good *In vitro* Method Practices (GIVIMP) details a set of quality standards to improve both the quality of and confidence in newly developed, and routinely executed *in vitro* methods. An important part of (and a source of potential high variability in) any method is the test system. Microphysiological Systems (MPS) have been used in novel methods for many years. Despite their potential benefits, there are several quality concerns associated with MPS. Two of these concerns are reproducibility and well defined and understood biological relevance, both of which are covered in the GIVIMP guidance. GIVIMP provides recommendations and points to consider intended to help improve the quality of the test systems used in *in vitro* methods. It is important that these MPSs align with the recommendations in GIVIMP to help support the reproducibility and relevance of the methods that use these test systems. Providers of MPS like 3D tissue culture and organ on a chip systems can reference the GIVIMP guidance as they set up processes and procedures to routinely prepare their systems for testing use.

Presentation: Poster

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Development of a vascularized osteochondral microfluidic model as a drug screening tool for osteoarthritis

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Introduction: As a whole-joint disease, osteoarthritis (OA) affects all joint components including the articular cartilage and the subchondral bone. In this context, it is of paramount importance to develop organotypic *in vitro* models to study the pathology and screen potential drugs. What follows describes a microfluidic model of osteochondral interface developed to mimic OA-like inflammatory conditions and investigate the effects of anti-inflammatory drugs. We looked into marker expression involved in cartilage degeneration, bone remodeling and angiogenesis in healthy, OA-like and drug-treated groups.

Methods: The model contains cartilage and bone compartments in direct contact. The cartilage compartment was realized by embedding articular chondrocytes in fibrin hydrogel. To model the bone, osteoclasts, osteoblasts, endothelial cells, and mesenchymal stem cells were embedded in fibrin hydrogel enriched with calcium phosphate nanoparticles [1]. The model was subjected to inflammatory stimulation with IL1-β and then used to test two different drugs: IL1Ra as an antagonist for IL-1β and Celecoxib which is a COX-2 inhibitor. On day 14, immunofluorescence was performed to assess the expression of different markers.

Results: Matrix metalloproteinases (MMP-1 and MMP-13) were selected as markers of cartilage degradation. As expected, MMPs were upregulated upon IL-1β treatment. However, they were not significantly affected by the drug treatment. The pro-angiogenic vascular endothelial growth factor (VEGF), was expressed mainly by endothelial cells in the control group, while it was expressed also by osteoblasts and osteoclasts in IL-1β treated samples. Interestingly, IL1Ra induced conditions similar to the control group, where mainly endothelial cells expressed VEGF. Differently, Celecoxib halted VEGF expression altogether. As for bone remodeling, Receptor Activator of Nuclear Factor-kB (RANK) expression by osteoclasts was significantly increased in OA group compared to the control. Both drugs decreased RANK expression, with the effect of Celecoxib being notably more than that of IL1Ra.

Discussion and conclusions: We demonstrated that the developed osteochondral model is responsive to inflammation and can be used to screen the effect of anti-inflammatory drugs on multiple biolog-

ical processes known to be involved in OA onset and progression, considering the complex crosstalk between cartilage and bone cells.

References
[1] Bongio, M. (2016). *Nanomedicine 11*, 1073.

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Immunomodulation in glioblastoma-on-chip

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Glioblastoma (GBM) is the most aggressive primary brain tumour. It has a very low survival rate due to the high tumoral heterogeneity and low therapy response. It has been shown that the tumour microenvironment (TME) has an indispensable role in GBM progression, supporting tumour proliferation and drug resistance development and suppressing the immune system efficacy [1]. Besides, the lack of adequate preclinical models mimicking tumour complexity makes developing new therapies a difficult challenge [2]. The advances in organ-on-chip technology allow the recreation of more physiological conditions, which will hopefully help decode the interactions between tumour cells and all the components of the tumour microenvironment.

We developed a COP-based microfluidic device consisting of a central chamber and two lateral channels without a physical barrier between them, allowing better co-culture models. Furthermore, since COP is an oxygen-impermeable material, cells embedded in collagen hydrogel and seeded within the central chamber only get oxygen and nutrients through the lateral channels. Therefore, molecule diffusion and cellular consumption lead to self-induced gradients within the central chamber, mimicking different metabolic areas within the tumour. This allows the simulation of GBM on chip and recreation of important characteristics of this tumour.

GBM TME is shown to be immunosuppressive, so we use our GBM-on-chip model to study the infiltration of donor-derived peripheral blood mononuclear cells (PBMCs) in different zones of GBM and their consequent modulation. However, our principal aim is to reveal the effect of mechanical characteristics of TME on tumour-immune cell interaction. We use collagen matrices of different stiffness to study the effect of mechanical factors on immune cell infiltration, proliferation or exhaustion. A better understanding of the mechanisms leading to immune suppression could help the development of more effective therapies.

References
[1] DeCordova, S. et al. (2020). Molecular heterogeneity and immunosuppressive microenvironment in glioblastoma. *Front Immunol 11*, 1402.
[2] Stanković, T. et al. (2021). In vitro biomimetic models for glioblastoma-a promising tool for drug response studies. *Drug Resist Updat 55*, 100753

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Design automation and simulation for microphysiological systems

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Microphysiological Systems (MPS), also known as Organs-on-Chips, are testing platforms that are utilized in academia as well as in the pharmaceutical, cosmetic, and chemical industries. They are composed of miniaturized organ tissues that are connected via a microfluidic channel network and, by this, emulate human or other animal physiology on a miniaturized chip.

Their design has become a considerably complex task and requires the orchestration of aspects like module size, shear stress, channel geometry, pump pressures, etc. Mastering this is a non-trivial endeavor. But still, most corresponding devices are mainly designed manually thus far. This frequently leads to designs that often do not perfectly work as desired after the first try, but require frequent (costly and time-consuming) iterations to refine the design.

Design automation methods can help here. They include, e.g., automatic module placement or channel routing – allowing to avoid many manual steps and generating at least parts of a microfluidic device (or a first approximation) in a push-button fashion. Subsequently, simulations (based, e.g., on *Computational Fluid Dynamics* (CFD) or higher levels of ion such as those provided by the so-called 1D analysis model) allow for validating the correct behavior of a design before even the first prototype is fabricated.

In this talk, we present design automation as well as simulation solutions for MPS designs. We review respective design steps and formalize a corresponding design specification based on them. We then apply established design automation solutions, which resulted in a method that generates an MPS design of the desired device. The suitability of the resulting design is then evaluated by using a combination of simulation methods that are inspired by real-world use cases and allow for a high level of detail and reliability while saving computational time.

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A novel, animal-free culturing setup for vascularized, 3D engineered muscle bundles

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Human cell-based, engineered skeletal muscle bundles (EMBs) are needed to reduce and replace animal models in biotechnological and pharmacological applications. Additionally, conventional myogenic media for primary and stem cells contain xenogeneic substances such as animal sera, raising concerns on batch variation and applicability of the obtained results to humans [1].

Due to the significant role of skeletal muscle in metabolism and energy consumption, vascularization is essential for EMB functionality. Human umbilical cord endothelial cells (HUVECs) are an often-used source for spontaneous forming of vascular networks *in vitro* [2].

Here, we aimed to establish a protocol for creating vascularized 3D EMBs in animal- or xenofree medium, using human satellite cells (SC) and HUVECs.

In EMB models, myoblast differentiation is often induced by serum deprivation, switching from 10% fetal bovine serum (FBS) to 2% horse serum (HoS) [1]. In our study, as potential replacements for FBS and HoS, Ultroser G (UG, an FBS substitute, Sartorius) and human serum (HuS) were tested first in 2D cultures and later in 3D EMB studies. Green fluorescent protein (GFP)-expressing HUVECs and SCs were cultured in a fibrin hydrogel in polydimethylsiloxane molds.

2D experiments suggest higher SC proliferation in UG and HuS compared to FBS-HoS controls. UG showed best cell attachment to culturing dish bottom. A similar trend was seen in the preparation of 3D EMB experiments, as the yield for cells expanded was higher in UG than HuS cultures.

In 3D EMB experiment, the GFP-HUVECs start to branch, align along the stretched bundle, and grow into the surrounding hydrogel within 3-5 days. After two weeks, the vasculature appeared functional in some SC containing samples. The crosscut samples showed lumen-like structures formed by GFP-HUVECs. Both the HUVECs and SCs were aligned along the direction of static tension. The maturity of myotubes will be determined through fluorescent skeletal muscle markers.

This protocol for 3D EMB formation appears promising for creating vascular EMBs. The animal-free culturing methods implemented should be further studied for their role in later myotube maturation.

References

[1] Cai, A. et al. (2018). *BMC Biotechnol 18*. doi:10.1186/s12896-018-0482-6

[2] Mykuliak, A. et al. (2022). *Front Bioeng Biotechnol 10*. doi:10.3389/fbioe.2022.764237

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Microfluidic multi-compartment perfusion device with integrated microelectrode arrays for neuronal research

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In this work, an open microfluidic perfusion device with integrated Microelectrode arrays (MEAs) is developed for neuronal research. It is known that neurological disorders are often caused by defective cell biological mechanisms that are tightly regulated in space and time. Neurons are unlike other cells in that they are highly compartmentalized and extend long processes over considerable distances and through varying extracellular microenvironments. The environment within these neuronal compartments is subject to strict control mechanisms for homeostasis. Investigations of these homeostatic processes must match the cellular organization in virtue of spatial and temporal resolution and possess the ability to not only monitor but also challenge a selected compartment. MEAs, often arranged in two dimensions with a uniform distribution of the microelectrodes, have been widely utilized for studies of extracellular electrical activities of neuronal networks under physiological and pathological conditions.

The microfluidic device features neuronal sub-compartment separated by a physical barrier which can only be passed through parallel microgrooves that guide the growth of individual axons. The multi-compartment device is made of Poly(dimethylsiloxane) (PDMS) which has been extensively used for organs-on-chip microfluidics devices. In addition, the complex device which holds structures of very different scales (µm, mm and cm) has reserved specific structures for patch clamping as well as inlets and outlets for perfusion tubes in both compartments. The custom fabricated MEA with 60 microelectrodes that are aligned with the microgrooves is introduced to the bottom of the microfluidic chamber to read out the excitation conduction along the axons. The microelectrodes are capable of either recording or stimulating neurons by accessing sub-compartment of a neuron and collect signals from the sub-compartment around each electrode simultaneously.

The system is finalized by reversible bonding 3D PDMS elements of microgrooves and compartments with the MEA where-

by the microgrooves are capped and optical access is provided. The medium flowing through the microgrooves and compartments of the chamber is controllable by external pumps to measure uptake and secretion rates of cellular sub-compartment as well as for compartment specific drug application under physiological and pathological conditions. The system is biocompatible, transparent, and consistent with miscellaneous microscopes.

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Improving reconstructed skin models by mechanical stimulation in organ-on-chip devices

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Physiological tissues are exposed to different mechanical stimuli in the human body. Mimicking these constraints in microsystems contributes to the establishment of more complex and functional organs-on-chip. Regarding the skin, the current *in vitro* models fail to reproduce the permeability characteristics of a native skin. According to previous study, the integration of a cyclic stretching function in a conventional protocol of reconstructed skin improved the maturity of the *in vitro* model.

Here, we have developed a novel microfluidic system that integrates a reconstructed skin under dynamic perfusion and mechanical stimulation. The device combines the integration of hyper-elastic and porous materials made of polyethylene foam, reproducing the air-liquid interface under mechanical stimulation. The hyper-elastic membrane deforms under the effect of a pressure generated by the actuation liquid. During its deformation, the hyper-elastic membrane comes into contact with and deforms the foam located above it without any leakages or alteration of the foam.

We showed thanks to an indirect cytotoxic assay that this non-conventional foam material is suitable for tridimensional cell culture. We have demonstrated that the resulting reconstituted skin was well structured by a dermis populated by homogeneously distributed fibroblasts and a correctly stratified and differentiated epidermis. Moreover, we have observed a good expression of filaggrin (biomarker of terminal differentiation) by keratinocytes in the superficial layer.

This well controlled stretching system could be a promising device for mechanical stimulation of a skin model and could potentially be used for other biological models. This work opens new perspectives for the design of new organoids on chip with integrated mechanical actuation.

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Modeling intrinsically weak blood brain barriers in CNS disease using the µSiM platform with patterned defects in nanomembranes

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The blood brain barrier (BBB) may become intrinsically "leaky" during neurodegenerative conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD), and even normal aging. Patients with these conditions are at risk for marked progression of their disease if they experience an episode of systemic inflammation with innate immune cells and pro-inflammatory cytokines targeting the BBB. We are interested in delirium superimposed on dementia (DSD), a serious sequela for older adults undergoing major surgery. Thus, we sought to establish a model with an engineered "leaky" barrier to study the response of an intrinsically compromised BBB to inflammatory conditions.

Our laboratory has recently created a tissue chip we call the µSiM (microphysiological system fabricated with a silicon nanomembrane) to model the human BBB using iPSC-derived brain-like microvascular endothelial cells (BMECs) and brain pericyte-like cells (BPLCs). The membranes we used to separate "blood" and "brain" compartments in the µSiM-BBB contain hundreds of millions of ~60 nm pores. We established reproducible protocols that achieve "tight" barriers between BMECs on these membranes (PeLY ≤ 0.5 cm/min) [1]. Our laboratory has also produced a dual-scale membrane with tens of thousands of micropores patterned in this nanoporous background [2]. Given evidence that endothelial cells respond to microporous substrates with morphological changes [2,3], we hypothesized that DS membranes could create an intrinsically leaky, but tunable, BBB model.

Consistent with our hypothesis, 5 µm and 3 µm DS membranes can lead to "leaky" barriers. We also found that 5 µm pores promote BMEC migration into the blood side of the chip whereas 3 µm pores restrict migration. Interestingly, the addition of BPLCs to the "brain" side of DS membranes appears to "rescue" the structural integrity of DS barriers. The essential role for pericytes as a structural cell may be consistent with the reduction of pericytes seen supporting the vasculature in DSD. Overall, this work provides a promising start to creating an intrinsically leaky but tun-

able barrier in the µSiM-BBB for the study of DSD and other CNS diseases.

References

[1] McCloskey, M. C. (2022). *Adv Healthc Mat 11*, 2200804.
[2] Salminen, A. T. (2019). *Small 5*, e1804111.
[3] Casillo, S. M. (2017). *ACS Biomaterials, 3*, 243.

Presentation: Poster

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Advances and challenges in generation of patient-derived tumor-on-chip (PD-ToC) models for time-efficient clinical decisions

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Introduction: One of the key challenges facing the field of tumor modeling is the need to transition from traditional cell lines towards patient-derived cell cultures that are more capable of accurately mimicking the complex *ex vivo* features present in the tumor microenvironment. We have developed a novel patient-derived Tumor-on-Chip model (PD-ToC), using rapidly isolated primary cells (cancer, immune, fibroblasts) from fresh tumor samples, incorporating the isolated populations into microfluidic chips, and following up with treatment using clinically-relevant therapies. PD-ToCs are then put under continuous live imaging allowing us to quickly and accurately assess *ex vivo* therapy response within these miniaturized 3D tumor ecosystems.

Results: We generated PD-ToC models from several patients with non-small cell lung or breast cancer, treated at the Bichat or Curie Hospital in Paris. Both CD8+ T lymphocytes (TILs) and matching epithelial tumor cells were then isolated from the tumor sample using magnetic-activated cell sorting (MACS) and encapsulated in collagen inside PD-TOC chips. The overall success rate of PD-ToC generation was around 75%. Death of tumor cells was quantified using the spatiotemporal apoptosis mapper (STAMP) algorithm [1]. For the vast majority of patients, in absence of treatment, the addition of primary TILs to autologous tumor cells led to increased cancer cell apoptosis, highlighting that the cytotoxic capacity of primary TILs is present and active in our PD-TOC models. The effect of chemotherapy (Paclitaxel) and/or immunotherapy (anti-PD-1) addition was also precisely quantified for each patient. Most importantly, using the PD-ToC model we were able to observe patient-dependent variation in immunotherapy response. The effects of co-culture with non-autologous primary CAFs could also be observed in the PD-ToC.

Conclusion: In our current work, we highlight the feasibility of PD-ToC modeling in both lung and breast cancer patients. However, additional challenges still remain with the timely incorporation of other supplementary cell populations (cancer-associated fibroblasts, endothelial cells, etc.) in order to generate fully autologous PD-ToC models. Moreover, appropriate patient cohorts will be needed in order to further evaluate the capacity of PD-ToC to predict clinical response in cancer patients.

Reference

[1] Veith et al. (2021). *PLoS Comput Biol 17*, e1008870.

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A vascular tumor-on-chip platform to decipher endothelial immunomodulatory function

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Introduction: The vascular compartment is a key player in tumor initiation and progression, not only as an oxygen and nutrient provider but also as a potential immunomodulator. Indeed, endothelial cells (ECs) regulate immune cells path from bloodstream to the tumor. However, most existing tumor-on-chip models lack vascularization. Here we present a vascularized lung tumor-on-chip platform to study the endothelial immunomodulatory function.

Methods: This PDMS chip presents a central collagen gel chamber containing a needle-guided tubular channel (200 µm in diameter) seeded with either lung cancer patient-derived ECs or commercially available HMVEC-L. The microvessel morphology and functionality have been validated by immunofluorescence, TNFα stimulation assay and permeability assay. Moreover, 43 endothelial genes have been rationally selected because of their known involvement in immunomodulation, and their expression have been

studied by RT-qPCR with high control in cell composition of the tumor microenvironment: with/without lung cancer cell lines or with/without primary cancer-associated fibroblasts (CAFs).

Results: We demonstrated that the lung microvessels generated with HMVEC-L or patients ECs formed a monolayer with tight junctions, as observed by VE-cadherin immunostaining and exhibited an effective permeability barrier to FITC-Dextran. Our experimental setup allowed transcriptomic analysis specifically on ECs by RT-qPCR. As expected, by TNFα stimulation, we observed an up-regulation of VCAM-1, ICAM-1, and E-selectin mRNAs, encoding proteins involved in immune recruitment. Interestingly, co-cultures of ECs with either cancer cells or CAFs induced a downregulation of VCAM-1, encoding a leukocyte adhesion protein. We are currently moving toward bulk RNAseq analysis, to go deeper in endothelial identity characterization and plasticity, upon co-cultures and drug treatments. We are enhancing the cellular complexity and biomimicry by introducing freshly-isolated tumor cells, CAFs, immune cells, as well as vascular flow.

Conclusion: These observations emphasize the discovery potential of our vascular tumor-on-chip platform to decipher the endothelial immunomodulatory functions and their modulations by the tumor microenvironment. Moreover, the use of patient-derived, possibly autologous, cells will increase the clinical relevance of this platform. This tool will be valuable also to study the impact of advanced anti-cancer drugs, like immunotherapy, on ECs functions and identity.

Presentation: Oral

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A novel 3D printed multi-component scaffold for targeted spinal tuberculosis therapy

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Introduction: Spinal TB is the most prevalent type of skeletal TB, accounting for over 50% of the cases. It disrupts the nervous system, resulting in permanent spine distortion. Early detection and the use of combination chemotherapy are essential for the treatment and cure of spinal TB. However, due to low patient compliance and the emergence of drug-resistant strains, current therapies have been linked to unsatisfactory therapeutic outcomes. Additionally, the growth of bacteria in the spine damages the bone, necessitating repair through surgery which leads to bone defects [1]. Currently, there are no effective strategies to achieve targeted-sustained anti-TB drug delivery and sufficient bone regeneration. Therefore, this research proposes the design and development of a dual-functional 3D-printed drug-loaded biomaterial scaffold that addresses local chemotherapy delivery and critical-size bone defects. This approach has the potential to overcome the challenges of bone regeneration and poor drug treatment of spinal TB in patients who have had surgery [2].

Aim: The study aimed to develop a novel multicomponent biomaterial scaffold conjugation using a 3D printing technique for the treatment of spinal TB.

Methods: The fabrication of the multicomponent scaffold consisted of a mixture of sodium alginate, polycaprolactone, polyvinyl alcohol, different concentrations of bioactive glass titanium, and bedaquiline as a model drug. The composition, structure, mechanical properties, and *in vitro* degradability of fabricated scaffolds were characterized. The cytocompatibility and osteogenic activity of the scaffolds were evaluated by *in vitro* cell culture.

Result: The results indicated that increasing the glass content of the scaffold, decreased its porosity and degradation rate. However, the compressive strength was enhanced. Furthermore, the incorporation of bedaquiline microspheres improved the cell adhesion, proliferation, and osteogenic differentiation of MG63 cultured on the surface of the scaffolds.

Conclusion: This novel formulation has potential to improve patients' healing time, therefore enhancing their quality of life.

References

[1] Ruparel, S. et al. (2022). Surgical management of spinal tuberculosis – The past, present, and future. *Diagnostics 12*, 1307.

[2] Pei, P. et al. (2018). 3D printed mesoporous bioactive glass/metal-organic framework scaffolds with antitubercular drug delivery. *Microporous and Mesoporous Materials 272*, 24-30.

Presentation: Poster

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Determination of respiration and acidification rates in dynamic cell cultures and organ-on-chips

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Constant monitoring of culture parameters like oxygen, pH, and glucose is needed to ensure physiological conditions in microfluidic cell cultures or micro physiological systems. Furthermore, the measurement of these parameters can give valuable information on the viability and metabolic state of the cultured cells. Despite the large improvement of the systems and stem cell technology in

the recent years there is a lack in the development of proper readout techniques for OoC. Especially, metabolic monitoring would be crucial to understand the cells response to different stimuli inside the chip. However, most systems do not offer possibilities for metabolic monitoring. Miniaturized luminescent sensors spots for oxygen and pH are integrated into various microfluidic cell and tissue culture devices using inkjet printing technique. The sensor spots in a size from 300 to 800 µm can be read out with a miniaturized multi-channel phase fluorimeter. Our sensors can be excited with red-light and emit light in the near infra-red range (< 700 nm). This suppresses background fluorescence or scattering from biological material. Various cell types were cultured in the different systems and their metabolic response was monitored using integrated sensor spots. Oxygen consumption rates and extracellular acidification rates of cells are assessed in stop-flow experiments. We perform standard mitochondrial and glycolysis stress tests using model drugs to assess the ATP production of human-induced pluripotent stem cells (hiPSC) inside a microfluidic chip. We perform similar assays in the Seahorse™ system for validation. This system is the gold standard for determination of cell metabolism in static culture. Both methods yield similar results in both assays. Similar methods are used to create a microfluidic assay for cell viability and nanotoxicity. In addition, the determination of respiration rates in a heart on chip system is shown. These results will pave the way for assessment of cell metabolism in complex microfluidic systems like Organ-on-Chips with the same precision and relevance as in the current standard for static cell cultures.

Presentation: Poster

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A vascularized glioblastoma multiforme within a 3D perfused microphysiological system: Combining a self-organized microvasculature and a central venule in a hydrogel

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The blood-brain barrier (BBB) hampers the development of innovative drugs and nanovectors to treat neuro-pathologies, as brain cancers. The most frequent and aggressive brain tumor is the glioblastoma multiforme (GBM). After diagnostic, the median patient survival is about 12 months. Less than 5% of diagnosed patients are still alive after 3 years. The 3D-Glimpse project develops an organ-on-chip-like *in-vitro* physiological microsystem, as an alternative to animal testing. The microchip is perfused to mimic the blood flow, and instrumented with biosensors for an integrated detection of nanocarriers transport. The project is divided into 4 objectives, with preliminary results and prototypes for the first 3 objectives.

1) Development of the BBB-on-chip. The combination of two methodologies allows the 3D coculture of human brain cells (HBMEC: human brain microvascular endothelial cells, HP: human pericytes, HA: human astrocytes). They organize as a vascularized tissue in a hydrogel of extracellular matrix (collagen microfibers and fibrin). The barrier function is validated under a physiologically pertinent shear stress.
2) Comparison of the BBB-on-chip in a healthy versus pathological context. The microchip is adapted to fit the microenvironment of a vascularized brain tumor, with adjunction of GBM cells (U87) to create a GBM-on-chip.
3) Instrumentation of the BBB-on-chip. Biosensing integration relies on functionalized piezo-electrical substrates (lithium niobate) for detecting the transport of nanocarriers through the BBB and a micro-processed porous membrane.
4) Screening of the therapeutic efficiency. Besides the transport, the nanocarriers will be screened for their delivery specificity, and their toxicity towards targeted and off-target cells. Dosage adjustments are expected to prevent the strong side-effects of those chemotherapeutic treatments.

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Transmission electron microscopy (TEM) study of *in vitro* cultured human placenta explants shows tissue remodeling

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Human placenta is a unique species-specific organ, which is ethically easily available *ex vivo*, and therefore, useable for toxicological studies. The Placenta Lab Jena is closely connected to the delivery room of the hospital and placenta donation is highly agreed. Small tissue biopsies (explants) are long-term cultured *ex vivo* in a microphysiological system to create a human based new approach method (NAM, [1]). The advantage of explants from placenta is the inclusion of all placental cell types in their natural 3D environment and the intact cell connection. The tissue is completely vital as it is taken immediately after birth. Compared to uniform cell lines or spheroids/organoids explants show intact cell layers of syncytiotro-

phoblast, cytotrophoblast up to vessels with endothelial cells, fibroblasts and fetal blood cells (mainly erythrocytes). Xenofree culture conditions allow conditions close to the *in vivo* situation [2].

To test cell and tissue behavior in toxicological studies, explants have been exposed to potential toxicants or drugs and analyses have been performed during and after the experiments with toxicological or medical supplementation. A setup with different concentrations of copper sulfate showed induction (low dose) as well as inhibition (high dose) of cell growth and changes of hormone expression and cell viability – leading also to the question of the influence of diet and environmental toxins during pregnancy. Transmission electron microscopic images of placenta explants exposed to toxicants have shown in detail time- and dose-related changes in tissue and organelle structure, senescence and remodelling under toxic conditions. As a next step some observations will be confirmed by immunohistochemistry and qPCR. In summary, human placenta explants have been shown to be a useful and sensitive tool in toxicological studies, supporting the applicability of human tissue in long-term culture as replacement for animal testing.

References

[1] Luconi, M. et al. (2022). Human-based new approach methodologies in developmental toxicity testing: A Step ahead from the state of the art with a feto-placental organ-on-chip platform. doi:10.20944/preprints202211.0206.v1

[2] Heger, J. I. et al. (2018). Human serum alters cell culture behavior and improves spheroid formation in comparison to fetal bovine serum. doi:10.1016/j.yexcr.2018.02.017

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A liver pre-metastatic niche model for the investigation of invasion of breast cancer on lab-on-chip platforms

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Primary tumor modifies the microenvironment of distant organs. These modified microenvironments, which affect metastatic processes, are called pre-metastatic niches (PMN). Primary tumors induce the formation of pre-metastatic niches via soluble factors and microvesicles they release. This study aims to model the liver pre-metastatic niche and investigate its effects on breast cancer cells.

Soluble factors and microvesicles released from the primary tumor were presented by conditioned medium (CM) from three different breast cell lines (MDA-MB-231, SKBR3, and MCF10A). The IC-chip (Invasion-Chemotaxis) comprising three parallel channels connected with three capillary burst valves were used. Liver cell (THLE-2)-laden Matrigel was loaded into the middle channel; CM or serum-free (SF) medium was loaded into one of the side channels so that the CM can induce the formation of the PMN in the liver; finally, breast cancer cells were loaded into the other side channel. Using this model, invasion phenotype and changes in gene expression for two metastatic breast cancer cell lines, MDA-MB-231 and SKBR3, were investigated. Invasion of the cancer cells towards the liver pre-metastatic niche model differed based on the cancer cell line and the conditioned media: The invasion capacity of both cell lines MDA MB 231 and SKBR3 was negatively affected in the liver PMN using generated by CM from SKBR3. The invasion capacity of the MDA-MB-231 increased in the presence of CM from MCF-10A, while there was no invasion effect on SKBR3. The invasion capacity of both cell lines was not statistically different from SF in the liver PMN generated by MDA-MB-231 CM.

RNA sequencing analysis showed that p53 and HIF-1 signaling pathways were up-regulated for the PMN generated by MDA-MB-231 and SKBR3.

Our results show that a lab-on-a-chip can be used to model the pre-metastatic niche, study its effects on invasion and complement data from analysis of gene expression.

We propose a model to be developed into a platform for the prediction of the metastatic behaviour of breast cancer cells and target tissues such as the liver.

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Seizures-on-chip to model human epilepsies

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Epilepsies are multifactorial neurological disorders, affecting over 65 million people worldwide in all age group. Epileptic seizures are defined as spontaneous abnormal transient discharges of neuronal activities in the brain circuitries. Unfortunately, the currently available anti-seizure medications (ASMs) do not control the seizures in one-third, that is, over 20 million of patients. The major reason for the lack of efficacy of current ASMs is the huge spectrum of mechanisms behind epileptic seizures. Further, currently used preclinical cell, tissue and animal models have inadequate validity to replicate the essence of human epilepsies.

We have taken several approaches to develop human cell-based models for epilepsy and seizure modelling *in vitro*. In general, we utilize human pluripotent stem cells (hPSC)-derived neurons and culture them on microelectrode arrays (MEA) [1] to study neuronal network activities both in normal and challenged conditions. We have shown that seizure-like activity can be induced with kainic acid [2], a known convulsive, to model both acute or more chronic seizure behaviors. Secondly, the seizure-like activity can appear endogenously in patient-derived cells. Thirdly, we have developed an advanced seizures-on-chip model, Modular Platform for Epilepsy Modelling (MEMO) [3], for more precise seizure initiation, spreading and termination studies. MEMO consists of unique three-compartment microfluidic chip, enabling the formation of functional brain-like circuitry of axonally connected neuronal networks. Integrated customized MEA enables the neuronal network measurements, seizure-like activity detection and drug responses analysis. Microenvironmental control by gas supply chamber enables prolonged MEA measurements and longer culture follow-ups. MEMO can be applied in the research of disease mechanisms, new drug targets and discovery of novel ASMs to treat epilepsies. It can also be utilized in seizure liability testing in drug development process generally. Thus, MEMO offers preclinical platform to accelerate the R&D processes of novel ASMs cost-effectively and helps to avoid the development failures of investigational drugs.

References

[1] Hyvärinen et al. (2019). *Sci Rep 9*, 17125.
[2] Mzezewa et al. (2022). *Stem Cell Res 60*, 102665.
[3] Pelkonen et al. (2020). *Biosens Bioelectron 168*, 112553.

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Disease modeling of impaired brain glucose metabolism using patient-specific iPSC-derived microphysiological models of the neurovascular unit

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Neurological conditions conquer the world; they are the leading cause of disability and second leading cause of death worldwide. Although there is growing evidence for the immense impact of disturbances in neurometabolism for overall brain function, only little is known about the underlying mechanisms (knowledge gap). Especially human insights are sparse due to a paucity of physiologically relevant model systems (research gap).

To address these challenges, we are developing a novel, human iPSC-based organ-on-chip model of the neurovascular unit (NVU) that integrates all neurometabolically active NVU cell types and specifically enables the inspection of neurometabolic coupling mechanisms. To categorically cast light onto the mechanisms behind impaired metabolism of glucose, the brain's principal energy supplier, we are focusing on an NVU-on-Chip disease model of glucose transporter 1 deficiency syndrome (GLUT1-DS). Since GLUT1-DS is monogenic, it presents an excellent paradigm to study cellular and molecular consequences of disturbed neuroenergetics, even beyond the disease itself.

We have successfully generated induced pluripotent stem cells (iPSCs) derived from fibroblasts of two GLUT1-DS patients carrying different mutations in the solute carrier family 2 member 1 (SLC2A1) gene. Moreover, we have differentiated neurometabolically relevant NVU cell types (endothelial cells, perivascular cells, astrocytes) as well as cerebral organoids from these GLUT1-DS-iPSCs and investigated (i) key cell type-specific characteristics as well as (ii) changes in energy metabolism compared to neural cells/organoids derived from apparently healthy iPSCs. To address shortcomings of existing NVU-on-Chip systems, we developed a novel three-compartment microfluidic platform allowing for advanced neurometabolic coupling while at the same time retaining accessibility of the individual compartments. Thereby, we can specifically study perturbations in energy metabolism, blood-brain barrier integrity and neuroinflammation as a consequence of GLUT1-DS *in vitro*.

Our GLUT1-DS-NVU-on-Chip model holds great promise to provide novel knowledge on the underlying mechanisms and pathophysiology of GLUT1-DS, and thereby not only benefit those afflicted by the orphan disease but impact our understanding of a variety of other CNS and metabolically linked disorders.

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Development and characterization of a breathing primary human bronchial epithelial model grown on the AlveoliX lung-on-chip system

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In recent years, advancements in engineering and cell biology have enabled increasingly complex and physiologically relevant *in vitro* models of human biology. Compared to immortalized cell lines, primary cultures retain more of the tissue's natural characteristics, increasing the biological relevance. Recapitulation of relevant human biology can be further improved by including additional biological functions of the natural environment, such as the stretching of airway tissue cultures to mimic the breathing motion of the lung. In this study, a Lung-on-Chip (LoC) bronchial epithelial airway model was developed. Primary normal human bronchial epithelial (NHBE) cultures were grown at the air liquid interface (ALI), forming 3-dimensional, pseudostratified cultures with tight junctions, ciliated cells, and mucus producing goblet cells. These cultures were grown on the AX12, which contains flexible membranes that can be mechanically stretched to mimic the natural breathing motion. The NHBE airway model was characterized by monitoring the cultures' growth, cellular morphology, cell viability, and by measurements of tight junction barrier integrity using transepithelial electrical resistance (TEER). Once healthy cultures were established, the model was further tested by exposing the cultures to a known toxicant, cadmium chloride ($CdCl_2$). Cultures were exposed to 10 µg/mL, 100 µg/mL, or 500 µg/mL of $CdCl_2$ in the cell culture media for 48 hours with and without mechanical stretching.

The morphology of the primary NHBE cultures grown on the AX12 was consistent with the cobblestone appearance expected of these cultures. As airlifted cultures matured, mucus production and beating cilia were observed within the expected timeframe, approximately 10 days and 14 days post airlift, respectively. By day 7, TEER values reached the benchmark of ≥ 200 ohms*cm^2, indicative of healthy, tight barrier function. Exposure to $CdCl_2$ elicited a concentration-dependent decrease in barrier integrity and cell viability that was not affected by the presence or absence of mechanical stretching.

In summary, we have developed a novel LoC NHBE model that demonstrates many features of the human upper airway. The morphology, maturation timeline, barrier integrity, and response to a known toxicant of the cultures were consistent with expected outcomes.

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Defined neuronal-astrocytic interactions enabled with a 3D-printed platform

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Developing treatment strategies for neurological disorders requires understanding fundamental cellular mechanisms and interactions. For example, recent studies found that astrocytes have a crucial role in neurological diseases and affect the function of neurons [1]. However, such investigation is challenging due to the complexity and the limited *in vivo* accessibility of the human brain. Conventional models based on 2D cell *in vitro* cultures help study the interaction between neurons and astrocytes but lack 1) a 3D environment and 2) controlled interaction points between cells of different types that are present *in vivo*. Recent 3D printing approaches partially address the first problem, but the resin autofluorescence and the limited control over cell positioning hinder their potential [2].

To address this limitation, we propose a solution based on a non-autofluorescent resin and a two-step seeding approach to develop a 3D-printed co-culture platform with defined neurite guidance and neuronal-astrocytic interaction points. Using two-photon polymerization and the commercially available resin IP-Visio (Nanoscribe, Germany), we 3D printed 2 x 4 pillar arrays (110 x 110 µm width and 100 µm height) on a glass substrate connected by 250 µm long neurite-guiding bridges. Additionally, neurite-guidance ramps connecting the top of the pillars with the substrate layer were added. Astrocytes are first seeded with low density (2500 cells/cm^2), resulting in the astrocytes being statistically seeded only onto the glass substrate. A subsequent seeding of neurons with high density (15,000 cells/cm^2) populates the top of the pillars and forms a co-culture with astrocytes on the glass substrate. The neurons on the pillars grow into a network following the guidance structures. The presence of neurite-guidance ramps allows the neurites to contact the underlying co-culture on the glass substrate. With this approach, we could isolate the contribution of contact-mediated interaction from biochemical signaling. The transparent resin is ideal for fluorescent-based analysis, such as calcium imaging and immunocytochemistry.

This platform can therefore simplify the study of the interactions between neurons and astrocytes in disease models or toxicological tests.

References

[1] Pekny et al. (2016). *Acta Neuropathol 131*, 323-345.
[2] Harberts et al. (2020). *ACS Nano 14*, 13091-13102.

Presentation: Oral

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An iPSC-derived microbiome-gut-brain axis on a microfluidic chip to model systemic effects of neurodegenerative diseases

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Recent evidence indicates that neurodegenerative diseases (NDs) are progressively observed in the context of alterations in intestinal physiology. Adverse shifts in the gut microbiome composition leading to intestinal dysbiosis, are now directly implicated in numerous NDs [1]. This effect is mediated by the microbiome-gut-brain axis (MGBA) which represents the bi-directional connection between our brain and gastrointestinal system. The vagus nerve serves as a direct route of communication as modulatory effects of microbiota are directly facilitated via vagal activation. To model and study the systemic effect of NDs on gut and brain health, we are recapitulating the MGBA by creating an iPSC-derived polyculture with vagal neurons and hippocampal brain organoids, followed by an intestinal epithelium with a functional microbiome, on a microfluidic chip.

For the cerebral component, iPSCs-derived neuroepithelial tissue was further specified into functional hippocampal organoids expressing relevant hippocampal makers such as Prox1, KA1 and Zbtb20 [2]. The vagus nerve was recapitulated by differentiating iPSCs into neural crest precursors which were then directed into functional, mature intestinal vagal neurons (VNs) [3]. VNs were distinguished by neuronal marker expression of TUJ1 and MAP2. PHOX2B expression verified intestinal vagal identity. Creating a stable co-culture of hippocampal organoids and VNs on a microfluidic chip lead to the spontaneous bi-directional sprouting of neural processes through µchannels in the chip, displaying synaptic connections and increased neural plasticity. With an outlook on the intestinal dysbiosis associated with NDs, human intestinal organoids (HIOs) were generated from iPSCs by following embryonal intestinal development. HIOs were defined by a typical polarized, columnar epithelium comprising relevant intestinal cellular subtypes such as enterocytes, goblet cells, Paneth cells and enteroendocrine cells. The microbiome could be modeled by introducing small intestine-specific and probiotic bacterial strains like *Lactobacillus acidophilus* to the intestinal epithelium. Prior to introduction, bacteria were live-stained to visualize their adhesion to the epithelial mucus layer and proliferation.

References

[1] Zhong, S.-R. et al. (2021). *Transl Neurosci 12*, 581-600.
[2] Sakaguchi, H. et al. (2015). *Nat Commun 6*, 1-11.
[3] Goldsteen, Pien A. et al. (2022). *Front Pharmacol 13*, 991072.

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Parametric design and manufacturing of mammary carcinoma chip by stereolithography for simulating drug transport around the tumor

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Progress in cancer treatment highly depends on accurate prediction of the efficiency of the various drug candidates including chemotherapeutic agents and micro/nano drug carriers. Utilization of preclinical models such as 2D cell culture and animal models fail to provide adequate information for effectiveness and safety of drugs due to inadequate recapitulation of the human cancer pathophysiology. In the meantime, microfluidic tumor chips offer a promising avenue for research and development of new disease models to evaluate the drug performance in physiologically relevant environments where parametric design of microfluidic tumor chips based on drug transport characteristics is essential for accurately simulating drug delivery. Moreover, Low-cost rapid prototyping of such systems to iterate through design ideas and parametrized geometry will significantly accelerate the progress in the field. To address this problem, this study introduces design and testing of a new tumor-on-chip based on a cost-effective, optimized stereolithography (SLA) based replica molding workflow using widely available consumer-grade 3D printing hardware and reagents. First, capabilities of SLA for microfluidics are investigated and optimal process parameters are determined for feature resolution, surface roughness and thermal durability of produced molds and devices. Features such as 50 µm width channels and < 30 µm wide partition spacings can be produced with the current workflow. Then, a multi-layer microfluidic device featuring two

PDMS channel layers is manufactured for 3D tumor culture. Top layer of the device features capillary supply and lymphatic drainage channels that are separated from the bottom compartment by a microporous membrane. Separation of perfusion and culture layers allow culture environments with varying complexity. In the meantime, variation of capillary-to-lymphatic area enables simulation of tumor progression that is marked by gradually diminished non-functional lymphatics. The capabilities of the device are tested by characterizing small molecule and nanoparticle transport across 3D culture of mammary carcinoma in collagen hydrogels, partitioned co-culture of different cell populations in tumor stroma, and cytotoxicity of doxorubicin. We anticipate that the findings from this study will be useful for recognition of transport oriented microfluidic designs and widespread adoption of SLA for prototyping of high-precision complex microfluidic devices.

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Developing a 3D blood vessel-on-chip microfluidic model of thrombosis

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Thrombosis, a major contributor to global disease burden, is regulated by the interplay between endothelial cells (EC), platelets and blood coagulation factors [1]. Understanding the underlying molecular mechanisms is of crucial importance for the development and management of anti-thrombotic therapies. However, few of the current *in vitro* assays include EC, a major limitation for research and translational studies. Here we present a vessel-on-chip model of thrombosis that includes all key components of haemostasis, namely EC, blood factors, blood cells, extracellular matrix and shear stress.

A needle-based fabrication technique was used to obtain a hollow cylinder (300 µm diameter; 5 mm length) embedded in a collagen matrix (based on [2]). Human umbilical vein EC (HUVEC) were seeded and cultured in the channel for 3-5 days, until a confluent monolayer was obtained, as confirmed by immunostaining for DAPI and VE-cadherin. Media turnover was obtained thanks to a custom, optically-accessible reservoir unit, allowing for monodirectional continuous perfusion. Permeability was measured as readout of vessel integrity, assessed by 70 kDa TRITC-Dextran diffusion test and imaging the vessel over time (8 min).

To investigate thrombosis, whole blood from healthy donors was perfused in the EC-lined vessels over 20 min. Image analysis and time-lapse microscopy were used to quantify platelet deposition and fibrin formation. Activation of EC with TNF-α (10 ng/ml, 4 h) resulted in increased platelet adhesion and fibrinogen deposition compared to control vessels, as expected. Endothelial activation was confirmed by qRT-PCR, which showed upregulation of adhesion molecules ICAM-1, E-selectin and pro-coagulation tissue factor (TF). Thrombus formation was inhibited by an anti-TF antibody.

To develop a personalised thrombosis model, we used Endothelial Colony Forming Cells (ECFC) from healthy donors [3], activated with TNF-α and perfused with whole blood as above. Platelet adhesion and fibrinogen deposition were comparable to HUVEC, supporting the feasibility of the approach.

In conclusion, we developed a perfused thrombosis model which combines all key elements of thrombus formation, including EC. The model is amenable to independent control over microenvironmental stimuli, crosstalk with tissue-specific cells, and the inclusion of patients' own cells and blood for personalised studies.

References

[1] doi:10.5482/HAMO-14-11-0075
[2] doi:10.1016/j.mvr.2006.02.005
[3] doi:10.3389/fmed.2018.00295

Presentation: Oral

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Optical glucose sensor for on-line and at-line measurements of MPS

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Glucose is the primary energy source of human cells. Therefore, monitoring glucose inside microphysiological systems (MPS) provides valuable information on the viability and metabolic state of the cultured cells. However, continuous glucose monitoring inside MPS is challenging due to a lack of suitable miniaturized sensors. Here we present an optical glucose sensor that is suitable for measurement inside microfluidic systems. It is based on the depletion of oxygen by the enzyme glucose oxidase in the presence of glucose. The changes in oxygen concentration are measured via oxygen sen-

sitive phosphorescent particles. Additionally, an inorganic catalyst is used to remove the produced hydrogen peroxide and enhance the stability. All components are immobilized in the sensing layer which is covered by a porous membrane that limits glucose diffusion. The sensor element can be miniaturized to a diameter of 1mm. It is fabricated, together with the reference oxygen sensor, onto a biocompatible, pressure sensitive adhesive tape for easy integration inside microfluidic systems. Both sensors are integrated in a microfluidic device which can be used as a Plug- and Play system with other MPS. The system was characterized under cell culture conditions (37°C and pH 7.4) for five days. The dynamic range of the sensor can be changed to 0-10 mM or 0-25 mM by the porosity of the diffusion barrier. The sensors can be operated at flow speeds that occur typically in MPS. The influence of dissolved oxygen concentration and pH level on the sensor response was investigated. The sensors withstand sterilization methods like UV, plasma or Beta irradiation. The Plug-and Play system was used for at-line measurements of glucose levels in (static) cell cultures. Results were in good agreement with a commercially available off-line glucose sensor. In conclusion, we developed an optical glucose sensor that can be easily integrated in microfluidic systems and is able to perform stable glucose measurements under cell culture conditions. The system can also be used together with existing MPS for on- or at-line measurements. Furthermore, it is spectrally compatible with oxygen and pH sensors previously published by our group, enabling multiparametric readout.

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Cytotoxic effects of mesenchymal stromal cell-derived extracellular vesicles in PDMS-free lung cancer-on-a-chip

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Lung cancer is the most common cause of death from cancer with estimated 50% lethal prognosis in the first year after diagnosis [1]. Depending on the stage of lung cancer patients may receive different types of treatment, from surgery to radiation, chemotherapy and targeted therapy. Although various treatment options exist, improvements in drug delivery are still needed, which could increase their specificity and reduce the required drug concentration and thus reduce drug-induced side effects. Several studies suggest that mesenchymal stromal cell (MSC) derived extracellular vesicles (EVs) have several advantages to become modern drug delivery systems [2], however current *in vitro* methods are not well suited for EV delivery tests. Therefore, the aim of this study was to evaluate the effects of MSC-derived EVs on lung-cancer-on-a-chip (LCoC).

To that end, we have developed LCoC model using HPMEC (Primary Human Pulmonary Microvascular Endothelial Cells) to represent endothelium and stable cancer cell line A549 (Lung carcinoma epithelial cells) to represent cancer tissue. EVs were isolated from adipose tissue-derived MSC (ASC52TELO) and imaged using CD90-conjugated gold nanoparticles under transmission electron microscope and specific EVs proteins were characterized by Western blot. LCoC model was cultured under air-liquid interface (ALI) conditions for two weeks. Afterwards LCoC chip was exposed to MSC-EVs with and without cisplatin and cisplatin alone for 48 h and cytotoxic effects on LCoC model has been evaluated using cell viability tests CCK8, LDH and caspase 3/7 apoptosis test.

MSC-derived EVs do not cause cytotoxicity on endothelial cells, but in the LCoC model it shows cytotoxic effects. The results will be presented at the conference.

Research was financed from project nr. ERAF 1.1.1.1/20/A/124

References

[1] Duma, N., Santana-Davila, R., Molina, J. R. (2019). Non-small cell lung cancer: Epidemiology, screening, diagnosis, and treatment. *Mayo Clin Proc 94*, 1623-1640. doi:10.1016/j.mayocp.2019.01.013

[2] Sun, Y., Liu, G., Zhang, K. et al. (2021). Mesenchymal stem cells-derived exosomes for drug delivery. *Stem Cell Res Ther 12*, 561. doi:10.1186/s13287-021-02629-7

Presentation: Poster

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A reproducible human blood-brain barrier model (µSiM-hBBB) for *in vitro* studies of cognitive disorders

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Blood-brain-barrier (BBB) dysfunction is a hallmark of many central nervous system diseases that are growing in prevalence as populations age. With advances in microphysiological systems (MPS) and human induced pluripotent stem cell (hiPSC) technology, improved human models can be developed that recapitulate critical structure and function of the BBB. We recently introduced a modular version of our µSiM platform (microphysiological system enabled by a silicon nitride nanomembrane) that offers the simplicity and conveniences of Transwell™ culture while en-

abling high resolution and live cell imaging and rapid exchange of paracrine signals in a plug-and-play format [1]. We establish reproducibility on the µSiM using a BBB model in two laboratories (University of Rochester; University of Bern). Small molecule permeability and immunofluorescent staining indicated comparable junctional development and immune phenotypes of hiPSC-derived brain microvascular endothelial cell (EECM-BMEC) culture between labs. Further, a more physiologically-relevant coculture model was developed, consisting of isogenic-matched EECM-BMEC-like cells and brain pericyte-like cells (BPLC), termed µSiM-hBBB. The µSiM-hBBB was used to evaluate the function of pericytes at the BBB, which have key roles in barrier stabilization and are less abundant in diseased tissue. However, the exact nature of pericyte contributions is still not well understood. Dual-scale (DS) membranes, which contain micropores etched onto a nanoporous (NPN) background, were used to simulate the effects of aging by creating an intrinsic weakness in barriers. While tight barriers were formed by EECM-BMEC-like cells cultured on NPN membranes alone, DS culture required pericytes for proper barrier formation. The mechanism of this rescue was evaluated via analysis of extracellular matrix production, and the presence of N-cadherin and gap junctions, which would indicate cell-cell contact. Preliminary data indicates that pericytes contribute the bulk of extracellular matrix, suggesting stabilization may be at least partially due to reparation of the patterned defects in DS membranes via synthesis of a robust basement membrane. Thus, the µSiM-hBBB is a unique and reproducible MPS that enables investigation of cell-cell interactions by isolating true contact-dependent signaling from paracrine signaling. Future studies can elucidate mechanisms of BBB dysfunction using AD-derived hiPSCs cultured on DS membranes.

Reference

[1] McCloskey, M. C. (2022). *Adv Healthc Mat*.

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Scalable 3D human adipose *in vitro* model for its application in a multi-tissue metabolic disease microphysiological system

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Metabolic disorders including type 2 diabetes, obesity, and non-alcoholic liver disease (NAFLD) are highly influenced by endocrine dysfunctions. Adipose tissue is an endocrine organ and its maladaptive response upon metabolic disorders directly impacts other organs' homeostasis, including liver and pancreas. Conventional cell culture models lack complex multi-tissue interactions found in human physiology and are therefore limited in accurate disease modeling and predictive drug testing. Here, we developed a 3D human adipose *in vitro* model, with the goal to recapitulate lipid metabolism and serve as a cornerstone of a multi-tissue metabolic disease microphysiological system (MPS). Screening novel therapeutic opportunities in an adipose tissue-liver axis, or adipose tissue-islet axis, etc., will reveal promising avenues for metabolic disorders affecting the organ cross-talk.

First, primary subcutaneous and visceral white preadipocytes were expanded and aggregated in low-adherence 96 well plates. The adipogenic differentiation of the 3D subcutaneous and visceral spheroid models performed over 14 days could then be further cultured under (patho-)physiological insulin and glucose concentration for an additional 7 days. Lipid staining proved the accumulation of lipid droplets within the 3D spheroid models. The analysis of lipogenesis in the form of bound glycerol further confirmed the presence of lipids. Together with the tested lipolysis and glucose uptake, preadipocytes showed a successful and robust adipogenic differentiation potential, and the associated differentiated spheroids were functional with stable cell viabilities during the culturing process.

The presented human adipose model faithfully represents the hallmarks of lipid metabolism in a highly scalable format. It constitutes a one of the three central elements for building a multi-tissue MPS, including InSphero's already established primary human pancreatic islet microtissues and liver microtissues. This work completes the next step towards the development of a multi-tissue model of glucose and lipid homeostasis. We will be next establishing its application as a drug discovery tool to identify anti-steatotic, anti-obesity, and anti-diabetic drugs.

Presentation: Poster

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Novel body-on-chip system for quantification of compound kinetics, validated using positron emission tomography data

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Drug discovery is an extremely lengthy and expensive process with high rates of attrition. Small molecules with preclinical success that enter clinical trials only have a 7.6% likelihood of approval [1], indicating a need for better predictors of in-human success earlier in the pipeline. Micro-physiological systems (MPS) have become increasingly popular in drug discovery to try to reduce this failure rate [2]. The majority of MPS developed in recent years target organ-organ interactions and/or toxicity testing, with fewer developments in the field of pharmacokinetic predictions.

We have designed and tested a novel MPS capable of delivering drug-loaded medium across different organ compartments, with circulation and perfusion comparable to that in humans as measured by gold standard positron emission tomography (PET). Using this device, we have developed an MPS-focused approach to drug screening that incorporates biomimetic chromatography, MPS-derived kinetics, and validation using gold standard human *in vivo* PET data for the same drugs. Docetaxel and flumazenil were chosen for validation due to their use in PET pharmacokinetic studies in humans. High performance liquid chromatography can be used to detect nanogram quantities of both docetaxel and flumazenil, with limits of quantification (LOQ) of 12.3 ng/mL and 7.7 ng/mL, respectively. Additionally, biomimetic chromatography can predict plasma protein binding of small molecules with less than 10% bias ($r^2 = 0.911$).

Taken together, the new prototype platform has great potential for further development into a tool that can be used to reduce the attrition of the drug discovery pipeline by allowing better quantification of compound kinetics and, thus, enabling early termination of novel compounds with poor predictors of *in vivo* success.

LC is funded by a NC3R/Unilever studentship (NC/V001302/1).

References

[1] Hay et al. (2014). *Nat Biotechnol 32*, 40-51.
[2] Roth A et al. (2021). *Science 373*, 1304-1306.

Presentation: Oral

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Pneumatically actuated mechano-biological platform for cyclic cell stretching and compression studies

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Mechanotransduction is a process where cells translate mechanical signals into biochemical activity. Mechanical cues such as compression, stretching, and shear stress can affect cells directly at the protein level or through gene expression and further to cell morphology, orientation, focal adhesions, or differentiation [1-4].

We have developed a pneumatically actuated platform for different mechanobiological studies: equiaxial [1] and uniaxial [2] stretching and lateral compression [3] of cell cultures. In the platform, multiple parallel stretching units can be operated simultaneously inside an incubator. Stretching units can reach a strain up to 10% [4] and can be used in both static and cyclic modes. The platform enables portability utilizing a small microcontroller-based vacuum pressure controller and a vacuum battery that allows the stretching units to be transported between different equipment and laboratories without losing the membrane pre-strain.

In the stretching units, cells are cultured and stimulated on a thin silicone membrane (200 µm). Direct access from the bottom of the unit enables high-resolution imaging and fluorescent imaging with an immersion objective in an inverted microscope during the stimulation. Furthermore, optical properties of different silicone film materials (n = 7) were compared and studied extensively. For the selected silicone film, SILPURAN® 200 µm, we measured the insignificant autofluorescence and the smallest Full Width at Half Maximum (FWHM) of a Point Spread Function (PSF) of an imaged fluorescent nanoparticle. That means it causes the smallest z-directional aberration, and thus results in the best optical resolution. Therefore, the stretching unit enables high-resolution live-cell imaging with an inverted microscope, is suitable for long term cell culture, and provides portability.

The stretching units are fabricated using PDMS cast on to a 3D-printed moulds [3]. Compared to previous design, [1,2,4] the aim was to enhance the manufacturing process and reproducibility. The new design enables single cast of the stretching unit and support multi-cavity injection molding and large-scale production in the future.

References

[1] Kreutzer, J. et al. (2014). *Med Eng Phys 36*.
[2] Kreutzer, J. et al. (2019). *Biomech Mod Mechanobio 19*.
[3] Peussa, H. et al. (2022). *PLoS One 17*.
[4] Kreutzer, J. et al. (2017). *Proc MARSS 2017*.

Presentation: Poster

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Spatially resolved transfection on a microelectrode array for tissue engineering applications

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In our work, we take advantage of a CMOS microelectrode array chip (MEA) [1] to perform the spatially resolved electroporation of the cells grown on its surface. Due to the limited cellular area affected, electroporation with microelectrodes inherently has low toxicity, and a large range of pulse parameters can be modulated to improve the intracellular delivery of molecular cargos. Thanks to the ability to test different electroporation conditions on different areas of the MEA, large screening experiments designed to yield optimal electroporation parameters can be performed in parallel.

We used this method to define electroporation conditions that achieved dosage-controlled intracellular delivery of small fluorescent molecules, and delivery of large Cas9-sgRNA complexes with high efficiency and spatial resolution [2]. Using the same method, optimizing the transfection of primary fibroblasts with a mCherry-encoding mRNA resulted in 98% of the cells expressing the desired fluorescent protein without any sign of cell death. That transfection yield is the highest reported so far for an electroporation-based technology. Moreover, by sequentially introducing different fluorescent molecules or nucleic acids in the extracellular medium, and electroporating cells at different locations on the MEA, different patterns of cell phenotypes can be created. This way, we achieved high efficiency, spatially resolved transfection of primary cells with three different mRNA molecules encoding for different fluorescent proteins.

Our results showcase the promising applications that electroporation on MEAs can serve, such as screening of large libraries of molecules or the creation of engineered tissues on chip for drug screening applications. In this last application, our MEA offers the unique opportunity to precisely build complex and more physiologically relevant tissue models, while enabling their long term, label-free monitoring through the different interfacing modalities integrated in the chip.

References

[1] Lopez, C. M. et al. (2018). A multimodal CMOS MEA for high-throughput intracellular action potential measurements and impedance spectroscopy in drug-screening applications. *IEEE J Solid-State Circuits 53*, 3076-3086 (2018).

[2] Duckert, B. et al. (2022). High-definition electroporation: Precise and efficient transfection on a microelectrode array. *J Control Release 352*, 61-73.

Presentation: Poster

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An optimized GUTonChip model for intestinal absorption simulation

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Introduction: The intestine is involved in relevant processes such as intestinal absorption and transport. To study intestinal absorption the use of transwell inserts and the protocol previously reported, with modifications [1] may be consider as a static reference model. However, intestinal absorption is a complex process where different cell types (enterocytes, goblet or Paneth cells) are involved and exposed to a gut shear stress that affect the process. These characteristics are not completely represented in the static model but can be optimized in a GUTOnChip model.

The aim of this study is to use a microfluidic device as a cell culture platform in combination to microfluidic pumps to evaluate the optimization of simulating dynamic intestinal absorption *in vitro* compared to a static reference model, using a simulated digest.

Our approach: Caco-2 and HT-29/MTX cells were seeded (9:1) into transwell insert or apical compartment of microfluidic device (BEOnChip). Both devices were compared in terms of cell differentiation biomarkers (mucins, tight junctions and different defensins, as biomarkers of goblet, enterocytes and Paneth cells, respectively) after 7 and 21 days of growing. Moreover, after 7 days of culture, the microfluidic device was connected to an air-based perfusion system (Fluigent) under a gut shear stress and monitored for 4 h. For that a digestion blank obtained after the simulation of human gastrointestinal digestion using the *in vitro* Dynamic Digester developed by AINIA was used.

Conclusion: In conclusion, cell differentiation biomarkers are increased in GUTOnChip model at 7 days showing that this model is optimized compared to the reference model, Transwell insert at 21 days, showing more mucin and defensin-secreting cells. Moreover, GUTOnChip model connected with microfluidic pumps can be used as a model capable of simulating dynamic intestinal absorption *in vitro*, allowing to use a less diluted sample of the digested and therefore, to the *in vivo* situation.

Reference

[1] Panse, N. and Gerk, P. M. (2022). The Caco-2 model: Modifications and enhancements to improve efficiency and predictive performance. *Int J Pharm 624*, 122004. doi:10.1016/J.ijpharm.2022.122004

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Functional characterization of interaction of immune cells and 3D tumor spheroids in a microfluidic system

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Evaluation of novel therapeutics often fails to reliably predict severe complications in humans, especially when the immune system is involved. The reasons are inter-species differences in animal models or the lack of relevant *in vitro* systems that include immune cells. Moreover, existing *in vitro* systems mostly rely on static co-culturing of different cell types leading to overestimation of effects due to forced cell-tissue interactions. The incorporation of medium flow may help to mimic physiological conditions more closely.

To tackle current limitations of immune-competent cancer-on-chip approaches, we developed a microfluidic chip and operation concept, which prevents undesired immune cell sedimentation and cell accumulation. Continuous resuspension enables culturing of recirculating cells over several days. The Akura™ immuneFlow chip was designed to study flow-dependent recruitment of circulating cells to different microtissues (MT) via gravity-driven perfusion at high tilting angles.

The Akura™ immuneFlow platform features standard formats and was manufactured by injection molding. Up to seven preformed 3D tumor MTs (TuMTs) with PBMCs were co-cultured in each microfluidic channel. On-chip staining protocols enabled monitoring of the co-culture. The top-open approach allowed for removal of MTs and suspension cells for off-chip analysis.

Different TuMTs (HCT-116, N87) could be retained in the respective chambers and maintained viable at high tilting angles over three to six days. PBMCs could be kept in suspension for up to six days and were not activated by flow-induced shear forces. Unspecific stimulation of T-cells led to physiological expression of early and late activation markers (CD69 and CD25), which were detected by periodic collection of cells. Multi-photon microscopy imaging confirmed that PBMCs interacted with and infiltrated into 3D tumor MTs.

The simple design of the chip facilitates user-friendly operation and high-throughput implementation in cancer research and drug testing. The selected applications revealed differences between flow and static conditions, so that it can be argued that physiologically more relevant processes could be recapitulated. This work constitutes an important step towards device application as a tool for the evaluation of on-target efficacy and off-target toxicities of candidate immunotherapeutic agents.

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Mechanosensitive TRPV4 channel guides maturation and organization of the bilayered mammary epithelium

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Introduction: Biophysical cues from the cell microenvironment are detected by mechanosensitive components at the cell surface. Such machineries convert physical information into biochemical signaling cascades within cells, subsequently leading to various cellular responses in a stimulus-dependent manner. At the surface of extracellular environment and cell cytoplasm exist several ion channel families that are activated by mechanical signals to direct intracellular events. One of such channels is formed by transient receptor potential cation channel subfamily V member, TRPV4, that is known to act as a mechanosensor in a wide variety of tissues and control ion-influx in a spatio-temporal way.

Materials and methods: TRPV4 depletion was conducted on MCF10A or 184A1 mammary epithelial cells (purchased from ATCC) using siRNA. Actin cytoskeleton organization was analyzed from monolayer cultures, alongside with calculation of cell-exerted tractions utilizing traction force microscopy. Maturation markers for both myoepithelial and luminal epithelial cells were screened using immunocytochemistry and western blot analysis. 3D cultures were performed with both MCF10A and 184A1 mammary epithelial cells to analyze the formation and maintenance of mammosphere structures.

Results and discussion: Here we report that TRPV4 is prominently expressed in the stem/progenitor cell populations of the mammary epithelium and seemingly important for the lineage-specific differentiation, consequently affecting mechanical features of the mature mammary epithelium. This was evident by the lack of several markers for mature myoepithelial and luminal epithelial cells in TRPV4-depleted cell lines. Interestingly, TRPV4 expression is controlled in a tension-dependent manner, and it also impacts differentiation process dependently on the stiffness of the microenvironment. Furthermore, such cells in a 3D compartment were disabled to maintain normal mammosphere structures and displayed abnormal lumen formation, size of the structures and disrupted cellular junctions.

Conclusion: Mechanosensitive TRPV4 channel acts as critical player in the homeostasis of normal mammary epithelium through sensing the physical environment and guiding accordingly differentiation and structural organization of the bilayered mammary epithelium.

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Pharmacokinetic modeling of oral and intravenous modes of drug delivery in a pumpless microphysiological dual barrier model towards *in vivo/in vitro* translations

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Barrier tissues in a human body allow selective passage of molecules and two such barriers are the gastrointestinal tract (GI) and the blood brain barrier (BBB). Following oral drug administration, the GI plays a crucial role in regulating drug transport from the intestinal lumen into the blood. Once in the blood stream, the drugs targeted to the central nervous system (CNS) must pass the BBB. To understand the fate and efficacy of drugs for CNS disorders it is imperative to develop a microphysiological system to mimic the BBB and the gut. We have created a pumpless MPS system with two barrier tissues separating three distinct body fluid compartments, designed to mimic physiological transport of drug compounds through the GI tract into the blood and across the BBB into the CNS. This system enables modeling of multiple modes of *in vivo* drug delivery: oral dosing into the gut, intravenous dosing into the blood stream, and intrathecal dosing into the CNS. We demonstrate functional barriers that maintain a transepithelial/endothelial resistance of > 1000 Ωcm^2 for the BBB and > 250 Ωcm^2 for the GI barrier within the systems, express GLUT-1 and Pgp transport proteins, and regulate the passage of drugs across the gut and the BBB, with focus on the CNS. The transport of three drugs, bicuculline, caffeine and kainic acid, administered into the device both orally and intravenously, exhibited differential transport consistent with *in vivo* differences, such that caffeine readily passed through both gut and BBB while bicuculline demonstrated restricted transport. The experimental pharmacokinetic behavior of these drugs *in vitro* was computationally compared to *in vivo* pharmacokinetics based on population PK (pop-PK) models. The model accounted for the intercompartmental clearance of the drug and drug specific binding of the compounds within the system. The ability of our device to mimic different modes of drug delivery into the blood and CNS with associated PK modeling makes this device a useful platform for testing drugs for toxicity as well as efficacy.

Presentation: Poster

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Novel single- and multi-tissue chips for predictive pharmacokinetic applications

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Over the past decade, substantial investments of government funding and venture capital have produced novel technologies of more intact systems that represent human organ physiology. These advances include cell culture systems that go beyond single cell type monolayer cultures, such as organoids, spheroids, and co-cultures in 2D and 3D formats, and tissue chip systems that can replicate several organ systems (liver, gut, kidney).

Human tissue chips, aka microphysiological systems (MPS), are traditionally microfluidic devices designed to recapitulate human physiology at the tissue level and enable long-term *in vitro* (co-)cultures. While current microfluidic-based tissue chips are primarily used in basic research, such technologies have limited utility in pharmacokinetic applications because the flow-through fluidic design, chip material, and small media & tissue volumes do not support drug quantification.

For this unmet need, we designed single- and multi-tissue chips for pharmacokinetics applications. Javelin chips are recirculating milli-fluidic chips made of low non-specific binding thermoplastic material. Our milli-fluidic chips provide larger tissue (> 200K cells) and media (> 1.5 ml) than microfluidic chips to enable multiple media sampling for kinetic data. The recirculatory perfusion system dramatically extends drug-tissue retention time allowing low-clearance and low-permeability drug studies.

We characterized each tissue (liver, kidney (proximal tubule) and skeletal muscle) functionality for 21+ days with single- and multi-tissue chips and demonstrated physiologically relevant levels of enzyme and transporter activity in order to conduct pharmacokinetic studies.

A diverse set of small molecule drugs from all ECCS classes with various clearance mechanisms was evaluated on single- and multi-tissue chips. We quantified on-chip pharmacokinetic parameters, such as hepatic metabolism, uptake & disposition, tubular secretion & reabsorption, and muscle disposition. These on-chip pharmacokinetic parameters were then successfully scaled to clinical parameters for IV drugs: hepatic clearance, renal clearance, and volume of distribution. The predicted PK parameters showed high correlation to clinical parameters.

This study demonstrated that this technology would offer an alternative to, and hopefully a replacement of, pharmacokinetic studies in laboratory animals for the purposes of understanding drug disposition in an intact mammal as a surrogate for human.

Presentation: Oral

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Towards physiologically realistic/relevant body-on-chip models; introducing organ-specific innervation

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The concepts of organ-on-chip and body-on-chip fall into spectrum of advanced microphysiological systems currently under huge research interest and great expectations as future tools. Today, there are several well-advanced single organ-on-chip constructs containing multicultures of human derived cells in microphysiological environments with integrated sensor technologies. Also, in body-on-chip area, multiple organs are connected in bodily order expressing systemic interplay between organs. Vasculature and innervation are two network systems present in almost all organs running throughout the human body. Of these, vasculature has been integrated to various organ and body-on-chip models whereas there is less progress in integrating innervation to these concepts.

In our Centre of Excellence in Body-on-Chip Research granted by Academy of Finland we have taken innervation as one of our main tasks. To overcome this challenging task, we use several approaches from static to perfusable microfluidics, 3D bioprinting, and to 3D cultures [1] as organ specific demands are different. So far, we have shown in microfluidic environment that human neurons create physical connections to human cardiomyocytes via axons and these connections enable functional interplay [2]. We have also created a model with integrated neuronal and vascular networks in perfusable 3D microfluidic chip. In more complex setting, 3D bioprinting was utilized to innervate human stem cell derived corneal stromal structure [3]. These current proof of concepts of innervation are further developed towards models addressing clinically driven questions in single organ and body-on-chip concepts. Additional work has been started with other organs as well to build innervation on those.

References

[1] Räsänen, N., Harju, V. et al. (2022). Practical guide for preparation, computational reconstruction and analysis of 3D human neuronal networks in control and ischaemic conditions. *Development 149*, dev200012. doi:10.1242/dev.200012

[2] Häkli, M., Jäntti, S. et al. (2022). Human neurons form axon mediated functional connections with human cardiomyocytes in compartmentalized microfluidic chip. *Int J Mol Sci 23*, 3148. doi:10.3390/ijms23063148

[3] Mörö, A., Samanta, S. et al. (2022). Hyaluronic acid based next generation bioink for 3D bioprinting of human stem cell derived corneal stromal model with innervation. *Biofabrication 15*. doi:10.1088/1758-5090/acab34

Presentation: Oral

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Using an *in vitro* neuromuscular junction model to investigate the effect of zilucoplan on functional impairment induced by AChR+ myasthenia gravis patient sera

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Myasthenia gravis (MG) is a rare autoimmune disease driven by autoantibodies targeting components of the neuromuscular junction (NMJ). Most autoantibodies target the nicotinic acetylcholine receptor (AChR), impairing neurotransmission through three mechanisms: AChR blockade, antigenic modulation, and complement activation. To dissect the pathogenic mechanisms of autoantibodies in MG, an *in vitro* NMJ model was established. This platform was used to examine the impact of anti-AChR autoantibodies on complement activation and neurotransmission, and examine the effect of zilucoplan, a peptide inhibitor via dual mechanism of action of complement C5 under clinical development for AChR-seropositive (AChR+) generalized MG. A microfluidic platform (NeuroMuscle™) was employed to connect motor neuron neurospheres and 3D cultures of skeletal muscle fibers to form functional NMJs. Functional connectivity was assessed with glutamate stimulation of neurospheres and subsequent calcium transients in GCaMP6-transduced muscle fibers. *In vitro* NMJs were incubated with MG patient sera in the absence or presence of zilucoplan followed by evaluation of Complement(C)5a/sC5b9 products, C5b9 deposition, and functionality. AChR antagonists confirmed functional connections of NMJ co-cultures developed in the NeuroMuscle™ platform. Sera from AChR+ MG patients, as compared to healthy controls, induced C5b9 deposition, led to a 5 fold increase in complement C5 split products, and reduced calcium transients significantly. Furthermore, addition of zilucoplan prevented membrane attack complex assembly and NMJ functional impairment. These data provide a mechanistic rationale for the clinical response observed in AChR+ gMG patients treated with C5 inhibitors and highlight the benefit of an *in vitro* human NMJ platform, to functionally dissect pathogenic autoantibodies and support drug discovery in NMJ-related diseases.

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Establishment of an *in vitro* immunocompetent skin model system for skin sensitization assay as an alternative to animal models

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A gold standard of the skin sensitization assay currently is the Local lymph node assay (LLNA), which offers 92% sensitivity and 78% accuracy [1]. However, LLNA assay is an animal-based assay and equips with many advantages, but it can only portrait one key event out of four which is the T-cells activation. Apart from LLNA, there are already other *in vitro* assays implemented into the OECD guideline [2]. But these assays share the same limitation with LLNA, which is the one key event detection [3].

Our goal is to develop a skin sensitization assay based on an immunocompetent skin model system with an ability to capture more than one key event at once to increase the sensitivity and accuracy of the *in vitro* skin sensitization assay. Monocyte-derived Langerhans (MoLCs) and CD4+ naïve T-cells were incorporated into a 3D full thickness skin model system. Then the immunocompetent skin model system was topically exposed to 2,4-Dinitrochlorobenzene (DNCB), Isoeugenol, Resorcino, Glycerol or inflammatory cytokines cocktail consists of 50 ng/mL IL-6, IL-1β and TNF-α as a positive control.

Following the exposure, MoLCs migration and maturation as well as CD4+ T-cells activation were accessed using flow cytometry method. Upon the exposure, MoLCs demonstrate a trend to migrate and upregulate CD86 expression in comparison to untreated control. Moreover, CD4+ naïve T-cells have shown a trend to upregulate CD69 expression as a response to the treatment. Although further evaluations of the immunocompetent skin model system are necessary, this immunocompetent skin model system has shown to be a promising assay to test skin sensitizers as an alternative to animal models.

References

[1] Tourneix, F. et al. (2019). Assessment of a defined approach based on a stacking prediction model to identify skin sensitization hazard. *Toxicol In Vitro 60*, 134-143.
[2] OECD (2010). Test No. 429: Skin Sensitisation: Local Lymph Node Assay. https://www.oecd.org/env/test-no-429-skin-sensitisation-9789264071100-en.htm
[3] Grundström, G. and Borrebaeck, C. A. K. (2019). Skin sensitization testing – What's next? *Int J Mol Sci 20*.

Presentation: Poster

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Microphysiological pipeline for reprogramming and differentiation – improving homogeneity and standardization

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Human induced pluripotent stem (IPS) cells are a powerful tool that provide an alternative to animal testing, in line with the 3R principle. Aside from the ethical implication of animal testing, switching to human cells in biomedical research produces more relevant results for human physiopathology, making drug and treatment development precise, faster, and cheaper. However, the use of iPSCs for therapeutics has been hampered due to the high material requirements, costs, necessity for a trained workforce, and low reproducibility on a large scale [1]. Furthermore, reprogramming and differentiation of cells usually result in a heterogeneous stem cell population, impeding the clinical application of cell therapy. To address this limitation, we utilized microphysiological technology to develop a simple protocol for reprogramming fibroblasts and their subsequent differentiation into ectodermal lineage on a Polydimethylsiloxane (PDMS) based microfluidic chip platform [2]. The suggested method was easy to use and cost-effective, with a reduced reagent volume and the number of input cells required. The iPSCs generated from our platform show the upregulation of pluripotency markers and the downregulation of mesenchymal markers. The fibroblasts reprogrammed into iPSCs were further neurally induced to yield neural stem cells on chip, using a previously reported protocol, and were compared with a line parallelly cultured in a conventional well plate [3]. These NSCs reported upregulation of SOX1 and NESTIN along with neuroectodermal markers such as PAX6, SOX1, and ZIC1, signifying their role in neural development. Finally, we performed bulk RNA sequencing on the NSCs produced on the chip and the ones produced parallelly in conventional well plates to highlight the influence of the culture method and the resulting transcriptomic differences between the two methods. This platform can further be used to differentiate into other cellular lineages, opening further avenues. Improving the transition of iPSC-based therapies from the lab bench to the patient's bedside reduces the cost and variability of the process and imparts standardization, yielding a safe and clinically relevant platform.

References

[1] Bender, E. (2021). *Nature 597*, S20-S21.
[2] Luni, C. et al. (2016). *Nat Methods 13*, 446-452.
[3] Lundin, A. et al. (2018). *Stem Cell Rep 10*, 1030-1045.

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Adaptation of organ-on-a-chip technology to BSL-3 environment: case study of SARS-CoV-2 infection on a lung-on-a-chip model

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Complex human physiological and pathological features allowed by organ-on-a-chip system (OOC) are particularly suitable to recapitulate human responses to infections and understand the mechanism(s) involved in their pathogenesis. The use of infectious agents that may be transmitted through the air and cause potentially lethal infections requires a specific highly secured environment and an adaptation of the OOC technology. To this end, we developed a Biosafety level 3 (BSL3)-compatible OOC system allowing us to study SARS-CoV-2 viral infection on a lung-on-a-chip (LOC) model. Human Small Airway Epithelial Cells (HSAECs) and Human Pulmonary Microvascular Endothelial cells (HPMECs) are cultivated under flow rate in the opposite side of a PET membrane in a PDMS-free chip. After cell proliferation, chips are cultivated on air-liquid interface (ALI) to allow epithelial layer to fully differentiate and recapitulate the complex tissue interface of lung. After fully epithelial differentiation, SARS-CoV-2 infection is realized for 24-72 hours in a BSL-3 laboratory adapted OOC system. This system consists of a hermetically sealed box with adapted electrical connectors, integrated pumping unit that provides flow within the chip. Mucus production, barrier permeability and tissue characterization are assessed to determine the fully differentiation of the LOC and the effect of SARS-CoV-2 viral infection. This BSL-3 laboratory compatible experiment system, tested with SARS-CoV-2 infectious model, opens new perspectives for the use of OOC technology in the study of highly infectious agents for different tissues. Results will be presented during the congress.

Presentation: Poster

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Assessing mitochondrial and autophagic changes brought about by memantine using the mitochondrial event localiser (MEL)

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Memantine is an FDA-approved, non-competitive NMDA-receptor antagonist that has been shown to have mitochondrial protective effects, improve cell viability and enhance clearance of Aβ42 peptide. Currently there are uncertainties regarding the molecular targeting as well as treatment concentration of memantine. Here we sought to investigate the concentration-dependent effects of memantine on mitochondrial fission and fusion dynamics, autophagy and mitochondrial quality control, using a neuronal model of CCCP-induced mitochondrial injury as a means of understanding how memantine aids in promoting neuronal health. GT1-7 cells were cultured under standard conditions, treated with a relatively high and low concentration of memantine (100 µM and 50 µM) and images were acquired using a Zeiss 780 PS1 platform. Utilising the mitochondrial event localiser (MEL) we have demonstrated the concentration dependent effects of memantine with both concentrations maintaining mitochondrial network volume whilst the low concentration in particular caused an increase in mitochondrial structure count as well as increased fission and fusion events following CCCP-induced injury. Additionally, we made use of a customised Python-based image processing and analysis pipeline to assess memantine-dependent changes in the autophagosomal and lysosomal compartments and have demonstrated the ability of both concentrations of memantine to increase the volumes of these structures. Following these findings, we investigated the possible role of memantine in inducing mitophagy. Taken together, our findings have shown that memantine is able to protect the mitochondrial network volume against CCCP-induced injury and that high concentrations of memantine are suitable to induce macroautophagy whereas low concentrations induce mitophagy.

Presentation: Poster

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Posttransplant lymphoproliferative disorder in the lung – development of an EBV infection model as a proof-of-concept test platform for EBV-specific T cell products

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For immunosuppressed patients, viral infections remain a major risk regarding their morbidity and mortality. Infection with Epstein-Barr virus (EBV) can lead to posttransplant lymphoproliferative disorder (PTLD) in patients undergoing immunosuppression after solid organ transplantation (SOT). PTLD results in uncontrolled proliferation of EBV-infected B lymphocytes which subsequently transform into tumorigenic B lymphocytes forming malignant lymphomas that often manifest in the lung. Treatment with new immunosuppressive agents has led to increasing incidences of PTLD in recent years with up to 10-15% in SOT recipients. T cell suppressive therapies prevent organ rejection efficiently but decrease the control of transformed B cells mediated by EBV-specific T cells and negatively impact disease progression. Current treatment strategies for PTLD are limited to antiviral agents and reduction of immunosuppressive treatment but require alternatives due to toxic side effects.

Adoptive anti-viral T cell therapy is a promising therapeutic approach that guarantees improved control of viral infection while simultaneously protecting transplanted organs. This novel therapeutic approach provides the possibility to reduce harmful consequences of PTLD by restoring the EBV-specific T cell response. Production of EBV-specific T cell products is already established in our hands. However, we aim to develop a suitable test platform to verify product safety and efficacy in a PTLD infection model. We have the unique opportunity to have access to primary lung material and blood from the same patient to build an autologous PTLD testing platform.

In preliminary experiments, we already established a human co-culture system consisting of 3D primary lung organoids and EBV-transformed lymphoblastoid cells (LCLs) simulating PTLD in the lung. A suitable culture medium for co-cultivation was identified and the interaction of LCLs with lung organoids was analysed. We are now adding autologous T cell products into the system. The final objective is the integration of the PTLD infection model into a multi-organ chip system, including e.g. liver, which allows the evaluation of safety and viral clearance by EBV-specific T cell products in the lung as a proof-of-concept test. This platform could then replace less suited animal models with a fully human autologous testing platform for all kinds of T cell products.

Presentation: Poster

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Recreating pathological endocrine signalling associated with PCOS using LATTICE, a tissue-agnostic multi-organ microfluidic platform

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During the menstrual cycle, the ovary dynamically produces sex hormones and releases oocytes. However, with Polycystic Ovary Syndrome (PCOS) follicles remain in an arrested state. This endocrinopathy affects up to 15% of females of reproductive age, presenting other characteristics such as ovarian hyperandrogenism, anovulation, and hyperinsulinemia.

To model the female reproductive tract and related pathologies there is a need for a microfluidic device that can recirculate and selectively transfer culture media between multiple large 3D *in vitro* cultures. This was addressed with the LATTICE platform, which uses stepwise flow through an open-well design.

Cultures relevant to PCOS were encapsulated in hydrogels (murine pancreatic islets; mPI) or placed in Millicell inserts (human fallopian tube explants; hFTE, murine ovarian explants; mOE). LATTICE mPI cultures produced consistent levels of insulin (n = 3, 68.12 ng/ml per islet), daily imaging and morphological staining showed no central necrosis and uniform cell distribution. Furthermore, a 13-day culture of mOE using gonadotropins induces lev-

els and dynamics of estradiol, testosterone, and progesterone found with a normal menstrual cycle (n = 3). Lastly, hFTE expressed oviductal markers, OVGP1 and FOXJ1, and cilia beating frequency was measured at 6.25 Hz. Interestingly, when exposed to high exogenous levels of androgens, this falls significantly to 0.54 Hz ($n = 3$, $p < 0.01$).

When increasing the ratio of luteinizing hormone (30 mIU/mL) over follicular-stimulation hormone (10 mIU/mL) we can induce features of PCOS in LATTICE mOE cultures. Histology showed arrested follicles with enlarged granulosa cells and high levels of progesterone indicate premature luteinization (11.6 ng/ml for PCOS vs 2.9 ng/ml for control). The hyperandrogenic phenotype was also present, with significantly increased testosterone production (12.2 ng/ml for PCOS vs 3.8 ng/ml for control, $p < 0.01$). Co-culture of PCOS mOE as an endogenous source of high levels of androgens with unidirectional flow to hFTE indicated a similar negative effect on cilia beating frequency, and more repeats are underway to confirm this.

In summary, LATTICE can support and recapitulate endocrine signaling relevant to PCOS. Future studies will use mPI and mOE cultures as endogenous sources for insulin and sex hormones to further study this pathology.

Presentation: Poster

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Anticancer potential of *Sutherlandia frutescens* in NCI-H69V small cell lung cancer mini-tumors

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Small cell lung cancer (SCLC) is the more aggressive form of lung cancer and accounts for about 15% of lung cancer incidences. Despite general advances in cancer treatment approaches, SCLC clinical outcomes remain poor due to increasingly low survival rates, patient relapse, and drug resistance. With chemotherapeutic interventions mostly limited to DNA-interacting agents like cisplatin and etoposide, the search for an improved alternative regimen is highly essential. Over the years, medicinal plants have been explored as a source of potential candidates for drug development, however, most traditional medicines remain poorly studied. *Sutherlandia frutescens* is a medicinal plant indigenous to southern Africa and has been used traditionally for the treatment of different diseases. While studies have demonstrated its cytotoxicity in certain cancer cells, more investigations are required to ascertain its anticancer and clinical potential. Studies have established the merits of the three-dimensional *in vitro* cell model over the two-dimensional flat cultures as it better represents *in vivo* conditions and tumor characteristics, and are therefore invaluable at predicting the pharmacological effects of drug candidates *in vivo*. In this study, NCI-H69V SCLC spheroids were developed using the clinostat-based rotating bioreactor system. Spheroid growth and viability were characterized over a 30-day period, and parameters including, planar surface area measurements, glucose consumption, soluble protein content, adenosine triphosphate (ATP), and adenylate kinase (AK) levels were determined. Validation to ascertain their suitability for anticancer drug screening was conducted by treatment with the standard drug irinotecan, for 72 h. Results showed a significant reduction in viability at established IC25 (248.2 nM) and IC50 (573.97 nM) concentrations compared to control spheroids. Similarly, *S. frutescens* recorded a marked reduction in growth, viability, ATP, and AK levels after 96 h at IC50 (0.498 mg/mL) and IC75 (1.207 mg/mL) concentrations, compared to the effects of irinotecan (IC50). In conclusion, NCI-H69V spheroid model was functional and valid for future anticancer studies. *S. frutescens* demonstrates significant anticancer activity and deserves further investigation.

Presentation: Poster

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Two-organ MPS with liver and heart tissues for early-stage drug evaluation

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We have developed a multi-organ MPS and operated it with primary liver and heart tissues that were scaled down 1:50,000 compared to their counterparts inside the human body. The platform is capable of recirculating extremely small amounts of cell culture medium (blood surrogate) – 100 µL to 200 µL – that represent the equivalent of the volume of human blood, scaled down by a factor of 50,000. The device operates with gravity-driven flow on a custom-made rotating platform and controls fluidic flow via passive flow controls. It delivers physiological fluid flow rates for both heart and liver tissue chambers (14.6 µL, and 26.4 µL respectively), as well as the remainder of the device: 59 µL of cell culture medium that flow through a separate fluidic loop that represents the remained of the body. The device also contains a mechanism to consistently replenish water that evaporates over time, typically about 10 µL to 20 µL every twenty four hours. Here, we co-cultured a mixture of primary non-parenchymal liver cells and primary hepatocytes (in the liver chamber) with cardiomyocytes (in the heart chamber) for 24 h to 120 h and demonstrated acute primary drug toxicity measurements. The device is also capable of identifying secondary drug toxicity to cardiomyocytes. Because of the passive nature of the flow controls many of the MPS can be operated in parallel, making it easy to systematically test large nanopar-

ticle libraries or drug candidate libraries in industrial settings. The devices are suitable for early-stage drug testing and for detecting secondary drug toxicity due to drug metabolites.

Presentation: Poster

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Inhibition of metalloproteinases extends longevity and function of *in vitro* aged human iPSC-skeletal muscle

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Cell culture longevity has long been a desired feature for *in vitro* chronic disease modeling and aging. The dynamic nature of certain cells, like skeletal muscle, contributes to their early peeling off the surface substrate and limits the system's ability to conduct long-term studies. This study aims to investigate ways to prolong cell-surface interactions in the culture of human skeletal muscle cells differentiated from induced pluripotent stem cells (iPSCs), through inhibition of matrix metalloproteinases (MMPs), the enzyme digesting extracellular matrix (ECM) components. It was shown that treatment of the muscle culture with the MMPs inhibitors, Tempol and Doxycycline, elongated the cell adhesion from ~30 days to up to 80 days, analyzed by phase microscopy observation. Functional testing indicated that inhibitor treated cells displayed significantly reduced fatigue index and a higher fidelity than untreated cultures. Culture treatment with an MMP inducer, phorbol myristate acetate (PMA), showed premature peeling of the myotubes, and reversal of the enhanced functional data induced by inhibitor treatments. Gel zymography data served as a proof of principle where cells treated with an inhibitor showed minimally active MMPs, while inducer-treated cells showed high MMP activity. All this data supports the idea that regulating ECM dynamics can serve to maximize *in vitro* myotube longevity. The result yields the possibility of more robust *in vitro* systems with downstream potential for more accurate long-term toxicology assessments and disease modeling.

Presentation: Poster

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Design and application of an adept aerosol/vapor lung-on-a-chip and aerosol/vapor delivery systems using toxic agents

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Organ-on-a-chip technology and other micro-physiological systems (MPS) were designed to recreate living tissues that mimic organ microenvironments through precise control of the cells, extracellular matrix, and other micro-environmental factors. While correcting many of the gaps present in traditional tissue culture with a more physiologically relevant model, these systems still suffer from limitations. The inability to accurately administer toxic aerosols to the lung epithelial cells is a specific limitation to current lung-on-a-chip technology. Having the capability to perform testing and analysis on tissues through conventional routes of exposure specific to the organ is paramount in achieving a complete biologically relevant system. To close this gap, we combined 3D printing technology with microfluidic organ-chip engineering to build a customizable open-top lung-chip specific for the evaluation of aerosol and vapor toxicity and efficacy testing. 3D printing technology was additionally used to design an aerosol/vapor delivery chamber specific to the open-top lung-chips. This approach overall allowed for customizable, time and cost-effective parts to efficiently optimize a novel aerosol and vapor delivery system for lung tissue exposures to super toxic agents. Overall, we designed, generated, and evaluated novel open-top lung-chip designs in this study that will be used to expand our capabilities for elucidating novel mechanisms of action, informing potential targets for future development of diagnostics, therapeutics, and medical countermeasures.

Approved for public release: distribution Unlimited

Presentation: Poster

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Autologous approach to develop an immunocompetent skin model using iPSC-generated fibroblasts, keratinocytes, and dendritic cells

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The development of immunocompetent skin models is significant for the *in vitro* identification of foreign skin sensitizing substances and can contribute in the sense of the 3R principles, to significantly reduce the number of animal experiments for skin sensitization tests. The skin sensitization assays recognized by the OECD are standardized but can only address one key event at the time [1-3]. Considering the complexity of the processes involved in the development of contact allergy, the question arises, as to whether this is adequately represented by a series of isolated test methods. Therefore, it is necessary to develop an assay that can offer full insight into the skin sensitization event and respond to the skin sensitizer the same way the physiological skin would. To develop such skin models, hair follicle-derived keratinocytes are transfected into induced pluripotent stem cells (iPSc) using Sendai virus based reprogramming vectors, each expressing one of the four Yamanaka factors. For the differentiation of iPSc into fibroblasts (FB), keratinocytes (KC) and dendritic cells (DC), embryoid bodies were formed and cultured in the specific differentiation mix for each cell type to be generated. A flow cytometry analysis to verify the marker expression of FB-/KC- and DC-associated markers as well as iPSc-pluripotency markers was performed throughout the differentiation process. To generate iPSc-derived skin models, iPSc-FB are added into an insert of a transwell plate and iPSc-KC are seeded onto that layer. Immunofluorescent staining could show the physiological similarity to human skin with a visible dermis and epidermis layer.

HFDK that were transfected with the Sendai-virus-Kit have turned into stem cell colonies. Furthermore, we could successfully reprogram the iPSc into fibroblasts, keratinocytes and dendritic cells, and incorporate iPSc-Fb and iPSc-KC into a skin model, with a dermis and epidermis layer. The next step consists of the incorporation of iPSc- DC into the 3D- skin model that can further be used for the *in vitro* identification of skin-sensitizing foreign substances.

References

[1] OECD (2020). Test No. 442C: In Chemico Skin Sensitisation. doi:10.1787/9789264229709-en

[2] OECD (1992). Test No. 406: Skin Sensitisation. doi:10.1787/9789264070660-en

[3] OECD (2018). Test No. 442D: In vitro Skin Sensitisation. doi:10.1787/9789264229822-en

Presentation: Poster

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A feasible model of *in vitro* adipose tissue for metabolic research

Thayná Avelino[1,2], Marta Garcia-Arevalo[1], Maiara Terra[1], Felipe Torres[1] and Ana Carolina Figueira[1]

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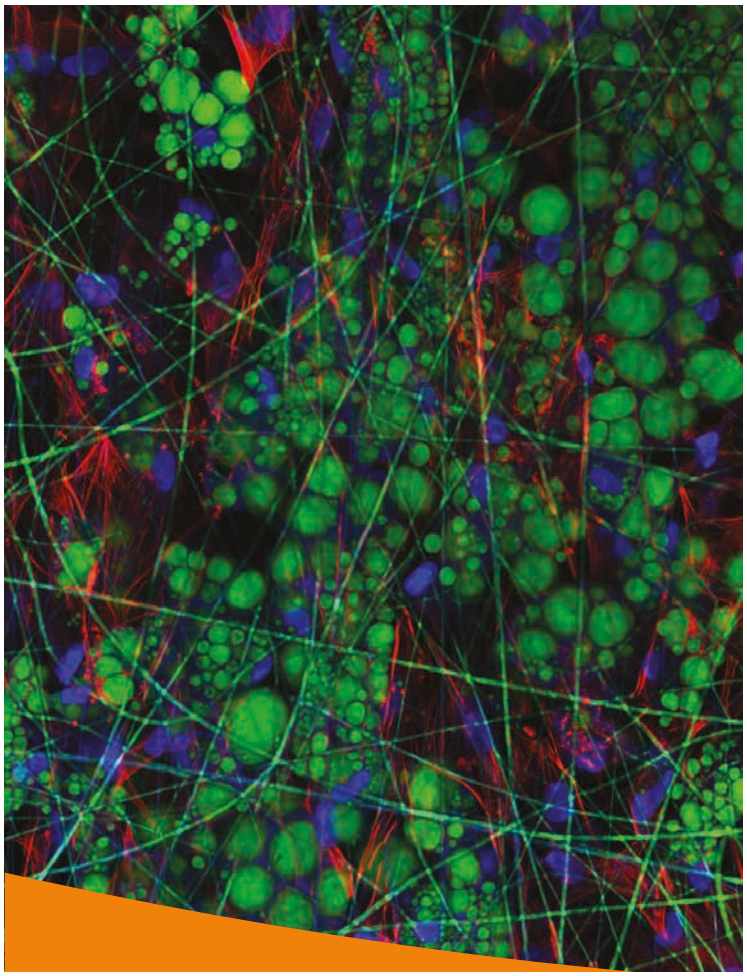
Worldwide obesity, defined as abnormal or excessive fat accumulation that may result in different comorbidities, is considered a pandemic condition that has nearly tripled in the last 45 years. In obesity context, adipocytes have a central importance, the role of adipocytes in energy storage is firmly established, only recently begun to appreciate the profound influence of adipocytes on other aspects of systemic metabolic homeostasis (Ghaben and Scherer, 2019).

The majority of studies on the origins of obesity uses animal models or adipocyte monolayer cell culture to investigate adipose tissue. However, in addition to monolayer cell culture approaches do not fully recapitulate the physiology of a living organism, there is a growing need to reduce or replace animals in research. Were found fundamental differences in cell behavior when comparing adipogenesis in 2D and 3D, due to effects on both cell morphology and 3D organization, supporting the idea that 3D cultures of adipocytes improve the recapitulation of adipogenesis and metabolic function in the spheroid model (Turner, 2015).

In order to reproduce *in vitro*, an adipose tissue model that mimic the environment where the adipocytes are metabolic more closely to *in vivo* adipocytes, we produce adipocyte spheroids with a workable cell (3T3-L1). To test our hypothesis, we differentiated the adipocytes and treated or no with Tumor necrosis factor-alpha (TNFα). To demonstrate the utility of the spheroids to study metabolic conditions, we compared the proteomic profile of our adipose spheroids with adipose tissue from lean and obese mouse C57BL/6J.

Take together our results indicating that we developed a simple, reproducible and low expensive three-dimensional model that recapitulate *in vitro* metabolic features of a real adipose tissue with an inflammatory and obesity profile, the development of these model could have considerable impact on the future of discovery for novel small molecules and biologics studies to prevent and treat metabolic syndrome and obesity in humans with the use of *in vitro* models.

Presentation: Poster



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TUESDAY | JUNE 27

11:30 AM – 1:30 PM
Session 1.1: Abstract 38

4:30–6:00 PM
Poster Session: Poster 127

WEDNESDAY | JUNE 28

10:00–11:30 AM
Poster Session: Poster 282 & 350

2:30–4:30 PM
Session 1.4: Abstract 332

THURSDAY | JUNE 29

10:00–11:30 AM
Poster Session: Poster 578

11:30 AM – 1:30 PM
Session 3.5: Abstract 630

4:30–6:00 PM
Poster Session: Poster 549 & 648

FRIDAY | JUNE 30

11:00 AM – 1:00 PM
Session 1.8: Abstract 568

1:30–3:30 PM
Closing ceremony: Keynote

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Human testicular steroidogenesis models for biomedical and toxicological research in a microphysiological setting

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With 7% of men suffering from infertility, health problems in male reproductive health and fertility have become a growing concern for researchers and the general public. Chemical exposure, drugs, radiation, and health conditions such as cancer are considered contributing factors to this alarming situation. Leydig cells play a crucial role in male hormone production and male phenotype determination, making them a relevant cellular target for these adverse factors. Moreover, it is known that an imbalanced sex hormone level, particularly during early life stages, is a key event in several adverse-outcome pathways (AOPs) leading to serious health problems such as impaired male fertility or Leydig cell tumors. However, current *in-vitro* models for exploring human testicular steroidogenesis for various purposes do not accurately reflect its biological complexity, species specificity, and developmental phase specificity. They mainly rely on rodent cancer cell lines, which lack the expression of a full set of steroidogenic enzymes, and their steroidogenic potential is limited. Induced pluripotent stem cell (hiPSC)-based models have the potential to revolutionize reproductive biomedical research and toxicity testing by successfully recreating Leydig cell development and functionality *in vitro*. My presentation will first overview the current status of human-relevant steroidogenic models with an emphasis on microphysiological systems. Secondly, I will present an approach to developing hiPSC-based models of Leydig cells applicable in biomedicine and chemical safety. Finally, these models will be compared to mouse embryonic fibroblasts and commonly used mouse Leydig TM3 cells in terms of morphology, functionality, steroidogenic potential, and human relevance. The ultimate goal of this study is to advance human-relevant non-animal methods for reproductive biomedical research and toxicity testing.

Research is supported by the Czech Science Foundation project No. GA22-30004S.

Presentation: Oral

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Engineering a microfluidic human tendon-on-a-chip to investigate inflammatory mechanisms in tendon healing

Isabelle Linares, Raquel Ajalik, Kaihua Chen, Benjamin Miller, Hani Awad and James McGrath

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Tendon injuries are among the most common musculoskeletal pathologies, accounting for over 30 million worldwide cases each year and a significant healthcare burden [1]. Tendon injury is characterized by a chronic inflammatory response leading to a fibrotic scar and failure to restore pre-injury joint motion [2]. While monocytes from the vasculature are thought to perpetuate this excessive inflammation, their biochemical and physical signaling roles in tendon healing remain unclear [3].

Previous work in our laboratory developed a human tendon-on-a-chip (hToC) that incorporates tendon and vascular compartments with patient-derived cells and a collagen hydrogel to mimic extracellular matrix. Ultrathin (< 100 nm) and highly permeable dual-scale membranes with nano- and micro-scale pores allow both cellular crosstalk between compartments and immune cell transmigration. While the hToC models a post-injury microenvironment in static culture conditions, a lack of physiological flow prevents the formation of a robust vascular barrier and circulation of monocytes. To address this, we describe the development of a plug-and-play microfluidic hToC to mechanistically investigate monocyte transvascular migration under flow and the downstream effects on fibrosis. We designed a flow component that is inserted into the existing hToC via pressure-sensitive adhesives, allowing for efficient assembly. To facilitate high-throughput experiments moving forward, the flow component will be mass-produced in collaboration with ALine, a microfluidic engineering company. Using COMSOL simulations, we have optimized flow conditions to reduce shear activation of circulating immune cells and attain physiological shear stress at the culture surface. Fluidic culture conditions established a more robust vascular barrier, as demonstrated by endothelial morphological alignment and enhanced glycocalyx expression. To investigate the impact of circulating monocytes on inflammation, we leveraged dual-scale membranes and compared them to nanoporous membrane conditions that restrict transmigration. Preliminary studies indicate monocyte infiltration into the tissue propagates a pro-inflammatory environment exhibited by increased inflammatory cytokine levels in the vascular compartment. Incorporating microfluidic capabilities into the hToC has enabled investigation of the vasculature's role in

fibrotic tendon pathology, addressing a critical need for physiologically-relevant tendon injury models.

References
[1] Nour, S. et al. (2020). *Regeneration*.
[2] Nichols, A. et al. (2019). *Transl Res*.
[3] Freedman, B. et al. (2022). *Nature Bio Eng*.

Presentation: Poster

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Reconstructing same-donor multiorgan physiology for studies of systemic immunity

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Some of our time's most significant biomedical urgencies come from metabolic and autoimmune disorders. Even though their clinical manifestation can often be associated predominantly with one particular region of the body, they involve the interaction of an intricate network of different cell types, organ systems, and a disruption thereof.

A notable example can be found in the gut-liver axis, where the two organ systems, and the environment, in the form of the microbiome, are intimately interconnected. Patients who suffer from inflammatory bowel disease (IBD) are more likely to develop certain inflammatory diseases of the liver and *vice-versa*. Significant correlations exist between ulcerative colitis (UC) or Crohn's disease (CD) and autoimmune hepatitis and primary sclerosing cholangitis (PSC). While interorgan crosstalk is most often discussed in the context of the cellular secretome and humoral communication, tissue-resident and migratory T cells are essential in orchestrating signaling between various tissues. An urgent need exists to understand the basic biology of how the mucosal T cell environment shapes systemic immunity in the context of autoinflammatory diseases and the role migratory T cells play in the multiorgan manifestation of these pathologies.

We have recently established a donor-matched MOMPS model of the gut-liver axis, which allows us to study TCR-dependent and independent activation of T cells across multiple organs and circulation of the same patient. We have streamlined the procurement of donor-matched liver, gut, and blood tissue and optimized the isolation of individual cell types of interest. These cells are being reconstructed on our multiorgan platforms at various levels of complexity and challenged via TCR-dependent and bystander activation. Using single-cell RNA sequencing, we have identified distinct clonally related T-cell subsets found in all three tissues with high effector and migratory capacity. MOMPS studies of their interplay with reconstructed intestinal and liver tissue further highlight how bystander T cell activation contributes to the perpetuation of inflammation in the gut-liver axis. A bioengineering approach to systems biology that comes with biological design and predictive human MOMPS can help us solve contemporary problems in biomedicine and to uncover casual relationships within complex biological phenomena.

Presentation: Oral

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Multi-niche human bone marrow on-a-chip for studying interactions of cell therapies with multiple myeloma

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Statement of purpose: Multiple myeloma (MM), a cancer of bone marrow-resident plasma cells, is the 2nd-most common hematological malignancy. Despite the advent of chimeric antigen receptor T (CAR-T) cells, relapse is nearly universally inevitable. The bone marrow microenvironment influences how MM evades treatment. Here, we present a multi-niche, microvascularized culture system to model MM behavior. This microfluidic human multiple myeloma on-a-chip (hMM-chip) is housed in a 96-well plate, making it compatible with automated fluorescence microscopy, liquid handling, and other data acquisition modalities. Furthermore, its network of endothelial cells encapsulated within the hydrogel enable the study of cell therapy trafficking within the tumor microenvironment.

Methods: The hMM-chip was assembled as previously described [1]. Briefly, a bottomless 96-well plate was covalently bonded to microfluidic channels fabricated in poly(dimethylsiloxane) and sterilized. Then, human mesenchymal stromal cells were differentiated into osteoblasts therein. Finally, an endothelial cell, stromal cell, and tumor cell-laden hydrogel was formed on top to form spatially-distinct bone marrow niches. The tumor cells were either cell line-based or primary bone marrow mononuclear cells from anonymous multiple myeloma patients from Emory's Winship Cancer Center. These live devices were imaged every 6 hours using an automated incubator-microscope setup to monitor fluorescently-labeled MM proliferation and localization to the vessel networks in real time. Additionally, anti-MM CAR-T cells were flowed into the devices, where they entered the gel through the endothelial networks and interacted with the encapsulated tumor, as captured by real-time *in-situ* imaging. Media was assayed for T cell activation cytokines and images of T cell-tumor interactions were quantified.

Results: Primary and cell line MM localized with the endothelium of the bone marrow's perivascular niche, as shown by micros-

copy. Further, the hMM-chip microenvironment was capable of supporting MM cells for at least 180 hours. Finally, T cells could be flowed into media channels of the device, and their anti-tumor effects were quantifiable through imaging of cell death and measured cytokine levels. Thus, the hMM-chip can nondestructively acquire various data modalities to study the effect of therapeutic T cells as they home toward and kill MM cells.

Reference

[1] Ghoshal, D. (2021). *Biomaterials 270*, 120683.

Presentation: Oral

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A human-based multiorgan microphysiological system for breast cancer metastasis modeling

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The prognosis for breast cancer in patients with metastasis remains poor. Breast cancer metastases are developed from cancer cells that originate from breast tissues and spread to the other part of the body through the circulatory system. Brain, lungs, liver, and red bone marrow are the four major sites of organ-specific metastasis for breast cancer. The aim of this study is to develop a human-based multiorgan microsystem to model the process of breast cancer metastasis that can be used for *in vitro* mechanistic studies and drug screening for the disease. We apply Body-on-a-Chip technologies by integrating 3D microtissues and circulating tumor cells into a recirculating microfluidic platform to simulate the dynamic interactions and metastasis of the circulating breast tumor cells to various sites. A multiorgan system was designed to include organ modules representing four main sites of breast cancer metastasis: liver, lung, brain, and red bone marrow. Chamber and channel dimensions were designed through residence time-based scaling to achieve physiologically relevant organ perfusion. The device consists of four main layers: the reservoir, perfusing channel, organ chamber, and bottom housing layers. The channel layers were made from polydimethylsiloxane (PDMS) replicas using 3D-printed negative molds. All other layers were fabricated from polymethyl methacrylate (PMMA) or silicone sheets and were patterned utilizing a CO_2 laser cutter. The fluid dynamics of the microfluidic chip was simulated in Ansys and validated with fluorescence bead flow experiments. The organ chambers were loaded with corresponding organ cells in hydrogel or as spheroids and maintained under dynamic circulating perfusion for 7 days. Fluorescent protein-expressing, metastatic breast cancer cells were added to the circulation through the reservoirs. Cell viabilities of all cell types and breast cancer distribution were characterized at the end of the experiment period. We demonstrated an integrated multi-organ microphysiological system to model breast cancer metastasis.

Presentation: Poster

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Improved frequency production of controlled microencapsulation using high viscosity polymer in low-cost centrifugal device

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Centrifugal encapsulation is a microencapsulation technique capable of generating monodisperse spherical capsules. Based on this technology, we propose an original method to increase further the production frequency of the microcapsules, while controlling the shapes and sizes, using a soft landing layer.

Introduction: Centrifugal microencapsulation can process polymer solutions with a large range of viscosity at high production frequencies while using possibly low-cost available lab material [1]. Microencapsulation has a variety of applications for drug screening, therapeutic and bioengineering applications that can offer 3D cell culture, organoids maturation or cell protection. Microcapsules formed with high concentrated alginate solution always present a protuberance [2,3]. As presented in our previous work [3], this important limitation is due to an insufficient relaxation time of the droplet during its flight in air before impacting the gelling solution. The original solution presented here consists in adding a layer of immiscible liquid on top of the gelation bath, slowing down the alginate droplet and thus increasing the time available for shape relaxation.

Experimental procedure: The microencapsulation device is composed of a conventional centrifuge tube with a disposable stainless-steel capillary connected to an alginate solution reservoir and a collecting calcium chloride ($CaCl_2$) gelation bath. When the device is centrifuged, the droplets formed at the capillary fly to the bath and travel through the $CaCl_2$ solution.

Results: After introducing an analytical model to evaluate the feasibility of the concept of soft landing layer, we present the modified centrifugal device and carry out an experimental screening of possible materials that can be used for the soft landing layer, in order to select the best candidates. An experimental parametric study was conducted and analyzed leading to an optimization of the device.

Conclusion: The experiments confirmed the efficiency of the concept presented here by producing controlled high viscosity alginate microcapsules at a frequency over several hundred per second.

References
[1] Haeberle, S. et al. (2008). *J Microencap 25*, 267.
[2] Jinbo, L. et al. (2022). *Chem Eng 427*, 130750.
[3] Badalan, M. et al. (2022). *Ind Eng Chem Res 61*.

Presentation: Poster

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An optimized kidney-on-a-chip model for handling graphene nanoparticles

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Nanomaterials have emerged in recent years as great tools for industrial and biomedical applications. Graphene, a carbon-based nanomaterial has been widely researched due to its excellent properties in terms of optics, mechanics and electroconductivity among others [1]. Advances in the application of these graphene-based materials (GBMs) in the biomedical field like drug and gene delivery, biosensors or tissue engineering have raised the necessity of testing their biocompatibility and their potential toxicity on the human body [2]. Due to their properties, nanomaterials like GBMs tend to deposit quickly, present low stability in aqueous solutions, and adsorb to plastic materials. Traditional approaches based on static assays facilitate their deposition and absorption and fail to recreate human physiological conditions.

In the present study, a kidney-on-a-chip model based on a microfluidic system under fluid flow conditions has been developed to solve these inconveniences and mimic the microenvironment present in human organs and tissues. Thus, the results obtained indicate that the adsorption of graphene-based materials depends on the materials used in the microfluidic setup. Alternative materials for tubing (PTFE and polymer-based on silicon materials) and devices based on PDMS or COP materials have been considered to grant the maintenance of initial nanoparticle concentration. Results obtained indicate that the adsorption and deposition of colloids depend on the materials used in the microfluidic setup, which significantly impacts colloid deposition, adsorption, and stability.

In conclusion, OOC models exposed to fluid flow allow to mimic human physiological conditions and provide an accurate model to assess exposure to nanoparticles and minimize their deposition.

References
[1] Lalwani, G. et al. (2016). Toxicology of graphene-based nanomaterials. *Adv Drug Deliv Rev 105*, 109-144. doi:10.1016/j.addr.2016.04.028
[2] Raslan, A. et al. (2020). Graphene oxide and reduced graphene oxide-based scaffolds in regenerative medicine. *Int J Pharm 580*. doi:10.1016/j.ijpharm.2020.119226

Presentation: Poster

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Steering epithelial and mesenchymal cell type composition in an iPSC-derived intestine-chip

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Introduction: Growth factor gradients along the crypt-villus axis define the spatial organization and diversity of intestinal epithelial subtypes in the human small intestine. Many intestinal model systems include diverse intestinal epithelial subtypes, however, not in a physiologically relevant quantity or location and often lacking the progenitor stages of these subtypes. Our aim was to replicate a growth factor gradient in an induced pluripotent stem cell (iPSC)-derived Intestine-Chip, hereby maintaining proliferating stem cells and inducing the diverse stages of differentiating epithelial subtypes. Additionally, we characterize the intestinal mesenchymal population upon exposure to this gradient.

Methods: Human intestinal epithelial and mesenchymal cells were generated from three control iPSC lines, which were then introduced in an Emulate Intestine-Chip. The cells were exposed to "expansion medium" mimicking the condition in the crypt region, "differentiation medium" mimicking the condition in the villus region or a gradient by introducing these media to the lower and upper compartment of the system respectively. The intestinal epithelial and mesenchymal populations were assessed via immunofluorescent staining, flow cytometry and single-cell RNA sequencing. Barrier integrity was assessed using a FITC-Dextran 4kDa translocation assay.

Results: We could steer the intestinal epithelial subtype diversity by changing medium composition. The differentiation medium (in one or both compartments) increased the number of goblet cells, enteroendocrine cells, enterocytes, and Paneth cells, however, the proliferating transit-amplifying cells and tissue morphology were better preserved upon exposure to expansion medium basolaterally and differentiation medium apically. Moreover, the gradient resulted in both progenitor and mature stages of epithelial subtypes, while having differentiation medium in both compartments yielded mostly mature subtypes. The mesenchymal population drastically reduced upon exposure to differentiation medium and was enriched in fibroblast-like subtypes. Further analysis of the single-cell RNA sequencing dataset

of the iPSC-derived Intestine-Chip will provide insight into the resemblance to reference data of the human intestine.

Conclusion: We present a thorough characterization of the intestinal epithelial and mesenchymal populations in an iPSC-derived Intestine-Chip. By applying a growth factor gradient, we obtain a physiologically relevant intestinal epithelial composition, capturing the entire differentiation trajectory from stem cells to intermediate progenitor stages and mature cells.

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Collaborative teams of biologists, engineers, and pathologists driving complex *in vitro* model engineering and characterization

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In 2022, US Congress passed the FDA Modernization Act 2.0, removing the legal requirement to use animal testing in drug development. This gives drug companies the option to use alternative testing models when making new drug submissions.

The European Society of Toxicologic Pathology (ESTP) and Society for Toxicologic Pathology (STP) are working together on the ESTP Pathology 2.0 Subgroup to bring together different scientists and increase the interdisciplinary communication between engineers, biologists, and pathologists in industry on the topic of Complex *in vitro* models (CIVM) and their use in drug development. Preclinical pathologists in industry are trained in comparative animal biology, which provides them with the expertise to bridge between animal models and humans. This knowledge can be expanded to CIVM. Nevertheless, pathologists need education and exposure to CIVM by the model developers and users, to increase confidence in morphologic evaluation and incorporating standard tissue technologies into CIVM experiments (i.e., histology, immunohistochemistry (IHC)/multiplexing IHC/spatial "omics"/high content imaging, electron microscopy, and automated image analysis algorithms). Examples will be shared of how those tissue technologies and analytics support CIVM engineering and its characterization/validation for their context of use. Overall, standardized pathomorphological evaluation, qualitative and quantitative scoring with deep learning models and robust histotechnical workflows handling CIVM will support accurate data generation and interpretation for assessing efficacy and toxicity in a reproducible, robust, sensitive, and high throughput manner. We will aim to show cross industry examples to facilitate exploration of these options for their projects as well. With this working group, we aim to make these CIVM platforms more translational between *in vivo* and *in vitro* and from animals to humans while growing collaborations, which are imperative to increase industry confidence in the use and application of CIVM for efficacy, mechanistic investigation, safety evaluation, and risk assessment in drug discovery and development.

Presentation: Oral

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A microfluidic multiorgan platform for automated cell culture utilizing iPS-derived cells

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In this work, we present a microfluidic system comprising a multiorgan chip and the respective control and readout instrument. The multiorgan chip has the following characteristics:

1. Standardized footprint (doubleslide) and fluidic interfaces (Mini-Luer) for easy interoperability with existing lab equipment
2. Manufactured by injection molding in transparent or black cyclo-olefin polymer (COP) for volume manufacturability
3. Contains four organ chambers with a suspended cell culture membrane in each chamber. One chamber is used to create a liver organoid, three for kidney organoids.
4. A microfluidic circuit connects the upper volume (above the cell culture membranes) of all four chambers for continuous perfusion. A second circuit in the lower part (below the membrane) of the liver chamber and a third for the lower parts of the kidney chambers are used for an automated medium exchange (typically every 24 h). In addition, each chamber has individual separate inlet/outlet channels for cell seeding.
5. Additional chambers and Mini-Luer connectors allow for the integration of sensor spots or sensor plugs to continuously monitor pH and O2 concentration in the fluidic circuits.

The associated instrument contains all elements to run long-term cell culture experiments, including fluidic controls (pumps, valves), temperature sensing and regulation, sensor readout, optical microscopy for cell growth control and a photomultiplier system for detection of luminescence signals.

With this system, we have been able to demonstrate seeding and fully automated culturing of iPS-derived cells, including sensor-based monitoring of growth conditions and a recording of reporter gene expression using reporter gene assays based on firefly luciferase [1]. The microfluidic chip was realised using industrial fabrication methods, allowing for a rapid transfer of research results into commercial manufacturing. Using standard sizes, interfaces and interface locations, the chip can easily be operated with a wide range of standard lab equipment.

This project was partially funded by the BMBF under contract 01EK1612A (micro-IPS-Profiler). We thank our partners from the Department of Nephrology, University Hospital Jena and Department of Biology, University of Heidelberg.

Reference

[1] Zuieva, A. (2022). Real-time monitoring of immediate drug response and adaptation upon repeated treatment in a microfluidic chip system. *Arch Toxicol 96*, 1483-1487.

Presentation: Poster

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Altering type I to type III collagen ratio for accurate microphysiological models of tendon injury

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The human-Tendon-on-a-Chip (hToC) is a collagen hydrogel-based microphysiological system (MPS) developed to model the fibrovascular-inflammatory interactions in tendon injury. Most *in vitro* collagen-based models of tendons such as the hToC use type I collagen. While type I collagen is found in healthy tendon, injury leads to dysregulated extracellular matrix composition with an increase in type III collagen. After injury, type III collagen may make up 20% of all collagens compared to less than 5% when healthy. The altered composition contributes to tendon scarring, poor mechanical properties, and structural disorganization of injured tissue. Directly controlling collagen ratios may bring this disease phenomena into MPS tendon models. We hypothesized that altering the ratio of type I to III collagen leads to differences in hydrogel ultrastructure that influence leukocyte migration in injured tissue models.

This was investigated using confocal microscopy and monocyte migration assays in collagen hydrogels made with type I and type III collagen. Levels of type III collagen ranged from 5% to 20% representing "healthy" to "injured" tissue. 3D images of collagen hydrogels stained with TAMRA-SE were processed to compare structural differences. As the level of type III collagen increased from 5% to 20%, fibrils became thinner and shorter making smaller pore networks. Monocyte chemotaxis experiments showed that cells moving through the 20% type III collagen gels were slower with more tortuous trajectories when responding to an fMLP gradient compared to cells in the 5% type III collagen gels. The mechanical and molecular transport properties of "injured" and "healthy" collagen are currently being evaluated using *in situ* microrheology and fluorescence recovery after photobleaching.

Changing collagen composition for *in vitro* tendon models leads to changes that resemble disorganized tissue seen in diseased tendon. The hindered migration of immune cells appears to be an important consequence of these ultrastructural changes. Anticipating additional impacts on local mechanics and molecular transport, MPS models should consider altered collagen compositions to accurately mimic the microenvironment of tendon pathology.

References

[1] Eryilmaz, E. et al. (2017). *Cell Mol Bioeng.*
[2] Fischer, T. et al. (2019). *Sci Rep*.
[3] Bzymek, R. et al. (2016). *Sci Rep*.

Presentation: Poster

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Breast tumor-on-chip applicable for efficacy and safety assessment of CAR-T cell therapy

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The success of chimeric antigen receptor (CAR) T cell therapy against solid tumors remains limited due to challenges associated with the tumor microenvironment (TME) and severe side effects such as cytokine release syndrome (CRS). To evaluate its efficacy and safety aspect, we developed a microphysiological breast solid tumor model on a chip that incorporates relevant TME components and allows constant perfusion of CAR-T cells.

The chip is specifically tailored to compartmentalize either ROR1-overexpressing breast cancer spheroids (MDA-MB-231 ffluc-GFP), fibroblasts spheroids lacking ROR1 expression, or patient-derived cancer organoids (PDOs) from patients' tumors with varying ROR1 expression levels. The tissue chambers containing the target cells are located underneath a media channel lined with primary microvascular or hiPSC-derived endothelial cells (autologous to CAR-T cells) formed on a porous membrane. ROR1-targeting CAR-T cells (or control untransduced T cells from the same donor) with an equal ratio of CD4+ and CD8+ cells were constantly perfused (20 µL/h) through the endothelialized media channel, where the T cells can extravasate and infiltrate the tumor. The platform was utilized to assess on-target on- and off-tumor cytotoxicity of CAR-T cells towards the abovementioned target cells via quantitative imaging analysis, monitor cytokine release kinetics (IL-2, IL-6, IL-10, IFN-γ, TNF-α, and granzyme B), and test dasatinib-mediated CRS intervention strategies during 8 days of chip culture.

Throughout the culture period of 8 days, we found that CAR-T cells infiltration into the tumor spheroids/PDOs and their cytokine response positively correlated with ROR1 expression levels. Their infiltration consequently hampered tumor growth. Additionally, cytokine levels and kinetics typically observed during CAR-T cell-associated CRS were recapitulated in the system. Incorporating dasatinib at different time points and duration during the chip culture enabled the modeling of CRS prevention, delay, and treatment, which consequently also resulted in varying efficacy profiles.

The ability to investigate the efficacy and safety aspect of CAR-T cell therapy and recapitulate patient-specific response in this model may provide new insights into the pathogenesis of CRS, test CRS intervention strategies, and ultimately find the balance between safety and efficacy of CAR-T cell therapy for solid tumors.

Presentation: Oral

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A 3D model for the survival niche of human long-lived bone marrow plasma cells

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Human long-lived plasma cells (LLPCs) are terminally differentiated effector cells of the B-lymphocyte lineage that reside in specialized niches in the human bone marrow (BM), harboring many different microenvironmental niches. LLPCs play an essential role in the humoral immune protection by maintaining constant high-affinity antibody levels against pathogens and their toxic products, independently of antigen presence. So far, the *in vitro* long-term cultivation of BM LLPCs is challenging since they reveal a brief survival time *ex vivo*.

Our previously developed scaffold-based 3D model can be used to maintain hematopoietic stem and progenitor cells for up to 8 weeks in their undifferentiated state (CD34+CD38-). Based on this data, we adapted the model to establish a microenvironment to support the survival of functional LLPCs *in vitro*. Human plasma cells (PCs) (CD38+CD138+) are isolated via magnetically activated cell sorting from femoral heads after mechanical preparation and introduced into the ceramic pre-seeded with MSCs building up a BM microenvironment. The survival capacity of functional PCs is assessed by flow cytometric analysis and detection of secreted antibodies by Bioplex.

Analyses of the niche microenvironment on transcriptomic level show that BM stromal cells are expressing PC niche relevant genes and molecules. Thus, a suitable microenvironment for the long-term cultivation of PCs is present. We are able to maintain viable PCs for up to 21 days when cultured with stromal cell contact, whereas PCs cultured without stromal cell contact show a very limited survival capacity. Upon stromal cell contact the cultured PCs in our system remain functional and maintain their ability to secrete immunoglobulins (IgG1, IgA, IgM) over the culture time. The mechanism involved in their survival within our model is still to be elucidated.

The established survival niche model could serve as a system to study niche interactions and will pave the way to establish disease models for diseases like multiple myeloma or autoimmunity to analyze changes in the microenvironment that promote the maintenance of pathogenic PCs. A better understanding of survival mechanisms of pathogenic PCs could disclose new targets for specific therapies.

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Advanced *in vitro* human airways models to study viral infections and perform antiviral drug screenings

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Advanced *in vitro* culture systems comprising epithelial cells derived from airway organoids and immune cells represent a valuable tool to study viral infection and pathogenesis. Here we describe two new human lung culture systems and exemplify their application to study host-pathogen interactions and drug screening.

First, we established a complex air-liquid interface (ALI) co-culture model consisting of human bronchial epithelial cells (hBECs) and blood-derived macrophages. This system was used to determine the role of different macrophage phenotypes (M0, M1, and M2) following respiratory syncytial virus infection. This study demonstrated that distinct macrophage subtypes responded differently to RSV infection, with M1-like macrophages significantly ($p < 0.0001$, $n = 3$) inhibiting the initial infection, whereas M0- and M2-like macrophages helped potentiate it. In addition, we found that the extent of these responses was influenced by the co-culture medium used in the infection assay.

Secondly, we generated hBEC-derived apical out airway organoids (Ap-O AO) using a serum- and ECM-free scalable workflow. This new 3D organoid culture model allows easy access to the apical side of the epithelium, enabling studies of host-pathogen interactions *in vitro*. Infection of Ap-O AO with influenza A, influenza B, rhinovirus-A16, or enterovirus-D68 produced high viral RNA titers (approximately 5.5 log10 copies per sample) and strong cytopathogenic effects, which were significantly decreased by the individual administration of two antivirals, rupintrivir and itraconazole ($n = 3$).

In summary, we showcase two new advanced airway *in vitro* culture models for studying infectious disease pathogenesis and performing antiviral drug screening.

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Heavy metals and metal mixtures elicit differential impacts on neurodevelopment in human 3D brain model

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Heavy metal exposure can impair neurodevelopment through disrupted cellular proliferation, differentiation, and network formation. While contaminants are regulated individually, exposures aren't isolated. To better characterize mixture toxicity on specific cell populations, we treated a human 3D brain model with Pb, Cr, As, and Cd alone and in combination during early (0-4 week) and late (8-12 week) differentiation in three independent experiments. For the first time in a BrainSphere model, we're also confirming intracellular bulk metal uptake with ICP-MS.

Following early exposure to AS, lineage-specific qPCR transcripts showed mild upregulation of NES (neuroprogenitors), SYN1 (mature neurons), and PLP1 (mature oligodendrocytes). No other condition elicited transcriptional changes; however, neurite outgrowth analysis indicated a mild reduction in neurite length and density among all early exposure conditions. In contrast, late exposure produced robust downregulation of MAP2, SYN1, and SYP and upregulation of NeuN (all mature neurons) among individual metals. Neurite length and density remained unchanged following mixture exposure despite mild increases with As, Cd, and Cr. GFAP and S100B (mature astrocytes) were upregulated following individual metal exposure. However, these results didn't extend to the metal mixture, suggesting an adaptive response or antagonistic effects of metals in combination.

We then investigated lineage distribution via flow cytometry. Early exposure produced heightened sensitivity, with individual metals producing a greater proportion of immature neurons and converting astrocytes compared to control, and mixture exposure inducing a slight increase in both immature and mature neuronal populations and converting astrocytes, and fewer NPCs. These results may indicate that NPCs are more rapidly differentiating upon exposure to a mixture of heavy metals, but that global transcript production is impaired. In contrast, late heavy metal exposure did not induce lineage distribution shifts, except for a slight increase in neuronal populations in the Cd group. Thus, altered transcription is likely real rather than a consequence of fewer neurons.

These results demonstrate the potential for heavy metal induction of neurodevelopmental perturbations at concentrations currently believed to be benign, and differential outcomes following mixture exposure. Early mixture exposure generally exacerbated the effects of single metals, while exposure later in development produced less sensitivity.

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Characterization of reproducibility and biological variability in a stem-cell derived human intestinal epithelium model for applications in inflammation

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Human primary cell-derived microphysiological systems have been a major focus of efforts to create more relevant models that enable enhanced preclinical predictive value of intestinal efficacy and toxicity. Transitioning from model development to model usefulness in stem-cell-based cultures has been challenging; the stochastic nature of stem-cell differentiation processes complicates the establishment of robust assays that query drug responses and pharmacokinetics. We aimed to identify and control sources of variability in a complex stem cell-derived intestinal epithelium model; multiple human donors, cell lots, and passage numbers of the isolated epithelium precursor cells were used in this effort.

Assessment criteria included metrics around barrier formation and maintenance (kinetic changes in transepithelial electrical resistance, TEER), gene expression, and cytokine (TNFα and IFNγ) response (LDH, IL-8, CXCL-11). Gene expression and culture metric analyses revealed that the upper limit on passage number of stem/progenitor cells required to minimize phenotypic changes, reduce variability, and maximize physiological relevance of the model is regionally dependent within the intestine. The use of transverse colon cells with optimized passage number in an inflammation-based case study allowed us to identify distinct cytokine responses from three human donors. Our findings demonstrate that it is possible to minimize extrinsic variability in complex culture systems such that experimental data consistently reveals an inherent biological response.

*Authors contributed equally: LMH & BZ

Presentation: Poster

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Investigation of the impact of gap scheduling on the toxicity of PARP1-selective AZD5305 combined with carboplatin using the bone marrow microphysiological system (BM MPS) and mathematical modelling

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AstraZeneca has developed a highly potent next generation PARP (poly-ADP ribose polymerase) inhibitor AZD5305 that selectively inhibits PARP1. The combination of AZD5305 with standard-of-care carboplatin is an exciting opportunity for the treatment of solid tumours, particularly because preclinically AZD5305 has minimal effects on haematologic parameters when compared with first-generation PARPi. Nevertheless, because carboplatin alone exhibits dose limiting thrombocytopenia and also impacts other haematopoietic lineages it is still critical to evaluate the potential for exacerbated haematologic toxicity when AZD5305 is combined with carboplatin.

Translating outcomes of preclinical models to clinical patients is a critical biopharmaceutical challenge and typically limited to animal studies. Our humanized *in vitro* bone marrow microphysiological systems (BM MPS) recapitulates the aspects of living bone marrow, thus can capture lineage specific haematotoxicity associated with oncology drugs. To achieve clinical translation, we integrate cell toxicity measured in the BM MPS into our MPS-based quantitative systems toxicology (QST) model.

We used this strategy to support the clinical development of PARP1-selective AZD5305 combined with carboplatin investigating concurrent and the potential for 24- and 48-hour gap schedules to mitigate haematotoxicity. We investigated clinically relevant exposures of carboplatin (AUC5/Q3W) and AZD5305 (at predicted human exposures from 1 mg and 10 mg QD dosing/14-days on/7-days off). Integration of clinical exposure profiles and MPS data into the clinical QST hematotoxicity model demonstrated that our predictions of peripheral haematotoxicity are in agreement with clinical data for carboplatin. Our results indicated that both concurrent and gap scheduling of AZD5305 in combination with carboplatin exacerbated (greater than additive) toxicity for erythroid progenitors and megakaryocytes respectively ($p < 0.001$ vs. monotherapy) in MPS. We then used the model to compare clinical schedules and predicted grade 3 (GR3) thrombocytopenia as a major adverse event with concurrent dosing and 24- or 48-hour gap schedule. Further investigation of efficacious low dose of AZD5305 and carboplatin or extended gap schedule may be required that could mitigate the haematotoxicity seen in combination.

Overall, our approach of using the preclinical MPS data and QST modelling to enable clinical translation is an industry first that help optimize clinical trials, reduces costs and, importantly, increases patient's safety.

Presentation: Oral

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Lung-organoid-infection models for preclinical testing of antiviral T-cells

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Respiratory viruses are the most frequent causative agents of disease in humans, with significant impact on morbidity and mortality worldwide, despite the availability of antiviral drugs. Patient-derived virus specific T-cell products (TCPs) represent a powerful alternative in the fight against viral infections. To better estimate outcomes and risk, preclinical testing of patient-derived antiviral

TCPs is required. However, current state-of-the-art models, such as non-physiological cell culture systems or animal models are only poorly suited for preclinical assessment, as their prediction fails in more than 80% of trials. Therefore, human organoid models represent a promising avenue for filling this gap, providing a more relevant human *in-vitro* platform, with the potential to reduce and replace less suited (animal) models. We have the unique chance to have access to iPSCs and PBMCs from the same subject, allowing the development of an autologous system. Thus, within the given project, we are pursuing the development of an iPSC-derived Influenza-A (IAV) Lung-on-a-Chip infection model for preclinical assessment of autologous IVA-reactive anti-viral TCPs. Here, we present the ground-workings of our iPSC-derived lung organoid platform and IVA-reactive anti-virial TCPs, which build the foundation for our preclinical Lung-on-a-Chip TCP investigation platform. We have successfully generated iPSC-derived human lung organoids and confirmed IVA infection upon apical-out inversion. Infected and non-infected organoids were subjected to single cell transcriptome sequencing and will be compared to infected and non-infected primary lung organoids. Furthermore, we successfully generated IVA-reactive TCPs from the same donor and show their IAV-specific cytokine production and killing potential in state-of-the-art *in-vitro* assays. At this stage, we are establishing suitable co-culture conditions, investigating antigen-presentation upon infection and adapting live imaging readout methods for our static lung organoid TCP investigation platform. We hypothesize that this platform will enable us to gain new insights into patient-specific adverse outcomes and better estimate safety and efficacy of virus-specific TCPs and can also be adapted to other viral or oncologic TCPs in the future.

Presentation: Poster

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Bioproduction of organoids and tumoroids in microbeads of human and animal extracellular matrices

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Organoids and tumoroids encapsulated in microbeads made of extracellular matrix (ECM) currently emerge as valuable models of *in vivo* physiological and pathological tissues [1]. Monodisperse ECM beads embedding induced pluripotent stem (iPS) cells or differentiated cells serve as very reproducible micro-environments supplying the 3D cell cultures with the necessary growth factors, cytokines, and chemokines while ECM porosity continuously provides them with cell medium nutrients and gas exchange. As a result, compared to organoid production in Matrigel domes, ECM beads of a few hundred micrometers in diameter are singularly tailored for massive and automated bioproduction in combination with an organoid biobank.

Bioproduction of ~275 µm-large (10 nL) droplets of various human and animal extracellular matrices was achieved at 2°C using a flow-focusing microfluidic chip [2]. First, pancreatic and prostatic cells were successfully laden in microbeads of Matrigel, a widely used ECM extracted from mouse sarcoma. However, due to its tumoral origin, Matrigel can hardly recapitulate native human microenvironment and is not compliant with Good Manufacturing Practices (GMP) [3]. Thus, human adipose tissue from abdominoplasty procedures, as well as porcine pancreas, were decellularized and delipidized to obtain decellularized ECMs (dECMs) more suitable for the culture of human cells. Every ECM droplet was reticulated into a microbead at 37°C in a CO_2 incubator which also provides the culture conditions for cell growth. Acinar organoids or tumoroids were formed inside the ECM beads within few days.

Advantageously, 3D cell cultures of few hundred micrometers in diameter are continuously maintained in suspension in their ECM bead microenvironment, allowing the non-destructive handling and possible immobilization of the organoids on hydrophilic treated surfaces in microwell plates and organ-on-chip assays. Growing organoids and tumoroids can be routinely characterized inside the ECM microbeads by optical microscopy and individually qualified by flow cytometry according to diameter and morphological criteria to achieve physiologically relevant tissues and disease modeling on a chip.

References

[1] Laperrousaz, B. et al. (2018). Nucl. Acids Res *46*, e70.
[2] Bottausci, F. et al. (2022). Proc EUROoCS 2022, P046, Grenoble, France, July 4-5, 2022.
[3] Papoz, A. et al. (2022). Proc EUROoCS 2022, P053, Grenoble, France, July 4-5, 2022.

Presentation: Poster

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Modeling pulmonary radiation injury using a human lung alveolus-on-a-chip

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Exposure to high levels of gamma radiation either due to environmental disasters or cancer radiotherapy to the thorax can result in development of acute radiation syndrome (ARS). The most fre-

quent acute pulmonary toxicity that arises is radiation-induced pneumonitis, an inflammatory condition characterized by increased neutrophil, macrophage, and lymphocyte infiltration, and subsequent fibrotic injury to the lung. Here, we leveraged a human lung alveolus-on-a-chip (Alveolus Chip) lined by primary human lung alveolar epithelial cells and pulmonary microvascular endothelial cells that form an alveolar-capillary interface and experience breathing motions to mimic acute lung injury in response to g-radiation exposure *in vitro*. Both cell types underwent DNA damage, cellular hypertrophy, upregulation of inflammatory cytokines (e.g., IL-6, IL-8, TNFa, TGF-b) and disruption of tight junctions leading to loss of barrier function within 6 h of radiation exposure; however, the endothelium exhibited greater susceptibility to radiation damage. Transcriptomic analysis revealed that radiation exposure enhanced the expression of cytoprotective genes including hemoxygenase-1 (HMOX-1) in both the lung epithelium and endothelium and network analysis identified HMOX-1 as a central mediator of radiation-induced injury. These findings demonstrate that the human Lung Alveolus Chip effectively recapitulates major hallmarks of acute radiation injury in the lung and suggest that HMOX-1 signaling pathway might represent a potential therapeutic target for development of novel radiation countermeasure therapeutics.

Presentation: Oral

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Implementation of customized 2D-to-3D microelectrode array designs: Optimization of passivation, electrode shape and methods for performance assessment

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Microelectrode Arrays (MEAs) are essential in neuroscience, enabling measurements of cultures of electrically active cells such as neurons [1]. Accurate studies of network-level phenomena require the determination of the exact location of recorded signals. Using electrodes with protruding shapes and caps increases the neuron-electrode contact area and improves electrical coupling [2]. To integrate these 3D-like electrodes into conventional planar MEA designs, the compatibility of fabrication methods must be ensured [3]. We introduce a unique design for 2D-to-3D MEAs including mushroom-shaped electrodes surrounded by an optimized passivation layer. This was achieved by implementing a dedicated fabrication process and incorporating suitable characterization steps throughout to evaluate the performance and quality of the microstructures. Si_3N_4//SiO_2//Si_3N_4 composite layers deposited with plasma-enhanced chemical vapor deposition showed the best performance, with a mean impedance of 34.3 ± 26.4 kΩ, obtained using Electrochemical Impedance Spectroscopy (EIS). Vias to the underlying planar MEAs were defined with Reactive Ion Etching. MEAs-chips with low impedances after etching (11.8 ± 6.8 kΩ) were selected for electrodeposition of gold and PEDOT:PSS to form the mushroom electrode. After neuronal culture growth, the noise level of all electrodes was analysed for individual electrode quality validation. A clustering algorithm based on fuzzy-c-means was implemented to classify recorded spikes according to the quality of the electrode-neuron coupling. Correlating the mapping of noise levels and spike shapes over the MEAs chip with SEM morphology inspection allows pinpointing sources of faults in the fabrication process, providing feedback for improvements. The implications of all implemented optimization steps and changes will be discussed within the framework and specification of neuronal activity recording.

References

[1] Teixeira, H. et al. (2021). *Adv Mater Technol 6*, 1-27.
[2] Mateus, J. C. et al. (2019). *J Neural Eng 16*, 036012.
[3] Tanwar, A. et al. (2022). *Mater Today Chem 26*, 101153.

Presentation: Poster

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Three human-derived hydrogels developed to support stem cell derived microphysiological systems

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Introduction: Hydrogels are three-dimensional scaffolds used as alternatives to *in vivo* models for the understanding of human disease. They can also serve as scaffolds, delivery system for cells and drugs, and as a 3D tissue system for drug screening. Here we are presenting three human-derived hydrogels, ObaGel®, ObaGel® ECM, ObaGel® Coating as alternatives to current murine-derived and synthetic recombinant hydrogels with unique physiochemical, *biochemical, and biological properties to support organ on chip.*

Methods: Three thermoregulated hydrogels were developed from human decellularized tissues through a series of processes optimized by Obatala Sciences. The human scaffolds were characterized for mechanical and biological properties using rheology, electron microscopy, and cellular proliferation and differentiation studies to be used as 2D basement membranes and as 3D systems. In addition to linage differentiation from Adipose derived stem cells, preliminary data on an adipose hypertrophy model is presented.

Results: Biochemical characterization provided differential levels of glycosaminoglycans and protein content. The manufacturing process was optimized to reduce lot-to-lot variability. Biocompatibility studies show a significant rate of proliferation and differentiation of the Stromal Vascular Fractions (SVF) and Adipose Stem Cells (ASC), supporting the role of human-derived hydrogels as scaffolds to promote differentiation into adipose and bone tissue and the utility of these matrices for the development of hypertrophic adipose tissue. Moreover, our studies prove the potential of hydrogels to supply a responsive metabolic environment, providing a platform for drug screening against metabolic disorders. Preliminary data for other applications will be presented.

Conclusion: This study provides the characterization of three hydrogels suitable for 3D culture and coating of microfluidic organ-on-chip devices, bioprinting, and regenerative medicine.

Presentation: Poster

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Using blood vessel-on-chip to characterize endothelial memory

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Background: Upon infection, innate immune cells and blood vessel cells (mainly endothelial cells) modulate the extent of inflammation, protecting the host against infection. While immune cells secret a lot of cytokines, TNF-α and IFNs are the two main signals stimulating endothelial responses. Recent studies show that innate immune cells can be trained to remember its exposure to pathogens, resulting in a stronger inflammatory response in subsequent encounters (trained immunity). In this project, we aim to investigate whether endothelial cells are also subjected to trained immunity and to characterize underlying mechanisms by profiling epigenetic changes in the cells. Using a blood vessel-on-chip model, we also aim to capture the effect of trained immunity on endothelial function in a physiological setting.

Materials and method: Primary endothelial cells (HUVECs) (from five donors) and hiPSC-derived endothelial cells (from three donors) were cultured in a 6 well-plate or in a viscous finger 3D vessel chip. Cells were stimulated once or twice with prominent inflammatory cytokines (TNF-α or IFN-y), with 1-5 resting days in between the two stimulation hits. Four hours after the last stimulation, cellular responses at RNA and protein levels were measured. To study the effect of repetitive exposures of endothelial cells to a stimulus on its interaction with monocytes, monocyte adhesion assay on viscous finger chips was performed. RNA-seq and ATAC-seq were also conducted to study the underlined mechanism.

Result: We observed a higher expression level of surface markers, secreted cytokines and higher number of monocytes adhered to endothelial cells that were exposed twice to stimuli in comparison with cells that were stimulated only once. Our data suggest that endothelial cells can remember previous exposure to cytokines and respond stronger in the next encounter. We also observed distinctive effects of TNF-α and IFN-y on primary endothelial cells and hiPSC-derived endothelial cells. There are also variations between cells from different donors. We are currently analyzing the epigenetic profile (ATAC-seq) and transcriptomes of cells under different stimulation conditions to explain the phenotypes.

The authors acknowledge the funding received from the Dutch Science Foundation (NWO) under the Gravitation Grant "NOCI" Program (Grant No. 024.003.001).

Presentation: Oral

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High-definition microelectrode arrays with scalable, integrated microfluidics in multi-well format for drug screening in a heart-on-a-chip application

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Towards increased throughput and automated workflows for organs-on-a-chip, a novel high-definition electrophysiology multi-well plate is developed in the Moore4Medical project [1]. It consists of an advanced CMOS microelectrode array (MEA) chip with 16 sampling areas, each featuring 1024 electrodes [2], and of a polymeric fluidic cartridge with 16 corresponding microchambers, each connected to an integrated pneumatic peristaltic pump [3]. It can perform 16-well assays with single-cell-resolution electrical recording and integrated microfluidics. Building on an earlier prototype, we present recent developments showing a scalable integration route and excellent fluidic and electrical functionalities of the plate in an easy-to-use system.

Direct bonding of the MEA chip and the cartridge was achieved using accurate glue deposition and a high-precision pick-&-place procedure, resulting in 16 well-aligned, sealed microchambers on top of the MEA. This method is developed for high-volume production. To run the plate, a user-friendly toolbox, which can be placed inside an incubator, is made together with a data acquisition unit, a pneumatic control unit, and custom software. The plate can be clamped directly inside the toolbox, ensuring communication of the MEA with the data acquisition unit by a pogo-pin connection. The pneumatic control unit actuates the 16 integrated pumps simultaneously via three pressure lines. Hence, 16 assays can run in parallel, with simple pipetting steps for cell seeding and media perfusion.

To demonstrate the plate's functions, all the 16 microchambers were primed and filled with water and 1% PBS buffer sequentially. No liquid leakage or bubble entrapment was observed. The voltage scan of the 16 MEA areas showed uniform signals and a clear difference between the two liquids (0.08 mVrms for water; 0.02 mVrms for PBS). Plate biological-validation is currently on-going to study the effect of drugs on electrical and contractile properties of human stem-cell derived cardiomyocytes and to demonstrate the plate potential for OoC standardization and workflow streamlining.

References

[1] https://moore4medical.eu/organ_on_chip

[2] Miccoli, B. et al. (2019). High-density electrical recording and impedance imaging with a multi-modal CMOS multi-electrode array chip. *Front Neurosci 13*, 641.

[3] Aksoy, B. et al. (2019). Latchable microfluidic valve arrays based on shape memory polymer actuators. *Lab Chip 19*, 608-617.

Presentation: Poster

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Complementing MPS with mechanistic computer models helps overcome limitations: Translating the drug exenatide from MPS to humans

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Today, realistic organoids can be combined into microphysiological systems (MPS), which are useful for a growing list of applications. Because of this combination, one can study dynamic cross-talk between organs, otherwise only observable in animals. This improved realism creates the potential to replace animal experiments, improve drug development, etc. However, using a purely experimental approach, there are certain limitations that are hard to overcome: the dynamic cross-talk makes data interpretation difficult, and today's MPS still display critical differences to humans, in terms of functionality, volume differences, and missing organs. We therefore propose to combine MPS with mechanistic computer models, which can help overcome these short-comings.

In this presentation, we demonstrate this potential for our two-organ MPS, with liver spheroids and pancreas organ model. Using this MPS, we can study central metabolism, in both healthy conditions and disease states, such as type 2 diabetes and liver steatosis. We develop the computer model by mechanistic hypothesis testing and validate the model by comparing experiments first done in the computer with subsequent MPS results. Using the validated computer model, we can also create more human-like versions of the MPS system. We can, for instance, scale the volumes of the organoids and the circulating media to the human proportions; this makes the consumption of a glucose tolerance test go from the unrealistic 48 h to the typical human timescale of 2-4 h. We can also add already developed computer models for the missing organs – muscle, fat, brain, etc. We demonstrate how this assembling into a

human model can translate MPS results for a specific drug: exenatide. MPS data shows improved insulin secretion, and predictions of human meal responses before and after exenatide treatment are validated by corresponding clinical data. Finally, we can also translate the MPS-based results of exenatide to mice, and from mice to humans, and thus include functions that cannot be observed in MPS, such as exenatide's impact on weight changes.

In summary, our integrated experimental-computational approach overcomes some of the inherent limitations of MPS and could help lay the basis for a new type of knowledge-driven drug development.

Presentation: Oral

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Compartmentalized culture of dorsal root ganglia on a soft thermoplastic elastomer chip: A proof of concept of an alternative to PDMS material for neurofluidics

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Compartmentalized microfluidic systems are a key enabler to study the nervous system while controlling its network structure and function. They are already mature for industrial applications, and they allow the expansion of new biological and co-culture applications, such as the study of the peripheral nervous system. [1] Most of the neurofluidic chips are made of polydimethylsiloxane (PDMS), presenting limitation (fabrication, industrial translation, and biological modeling). Easy to prototype and to translate, soft thermoplastic elastomers (sTPE) have emerged as an alternative material for on chip biology with a potential for cell biology to explore. [2]

Here, we develop the first compartmentalized device made of sTPE growing embryonic Dorsal Root Ganglia (DRG) in a tailored design and compare its performance to a standard PDMS. We emboss a FDA approved copolymer of polystyrene and ethylene/butylene and exploit its bulk properties for fast prototyping (less than 10 min/chip) and reversible adhesive bonding. It allows easy and accurate manual assembly of two-level architectures as well as direct analysis using atomic force or scanning electron microscopy. We demonstrate its capacities on murine embryonic DRG cultures in a tailored axisymmetric design compatible with high content screening microscopy. We cultured dissociated DRG and explants for more than 1 month in our devices with controlled convection, diffusion, and evaporation. We compartmentalized soma from the axons in ≤ 5 days. After a simple washing and sterilization procedure, we can open the chips and re-use them up to five times without impacting their bonding or the viability and axonal growth of the cells. The sTPE neurofluidic chip paves the way to an alternative way of modeling complex neural systems on chip, widening its field of applications and making its manufacturing more accessible and sustainable.

References

[1] Hyung, S., Lee, S. R., Kim, J. et al. (2021). A 3D disease and regeneration model of peripheral nervous system-on-a-chip. *Sci Adv 7*.

[2] Salmon, H., Rasouli, M. R., Distasio, N. et al. (2020). Facile engineering and interfacing of styrenic block copolymers devices for low-cost, multipurpose microfluidic applications. *Eng Rep 3*.

Presentation: Poster

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Metabolic and proteomic profiling of organophosphate chemical warfare agent exposure on CNBio human liver-on-a-chip

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Organophosphates (OP) are used as pesticides and chemical warfare agents (CWAS). OPs can range from moderately to highly toxic. Organophosphate chemical warfare agents (OP-CWAs) like Sarin (G-series) and VX (V-series) are common CWAs. OP-CWAs work by inhibition acetylcholinesterase, therefore disrupting neuronal signaling. Alongside inhibition of acetylcholinesterase, there are "off-target" effects. If common "off-target" pathways can be identified, this could allow for more effective broad-spectrum countermeasures.

One off-target effect seen in the literature is VX disrupting the TCA cycle [1]. This work set out to determine if G-series OP-CWAs created the same disruption in the TCA cycle via an *in vitro* human liver model. The CNBio liver-on-a-chip system was seeded with 600,000 cells/well and cultured for 6 days. On day 6, the cells were exposed to VX, Sarin, Soman, V1, or a DMSO control. After 24 h the scaffolds containing the cells were harvested and flash frozen, then stored at -80°C until processing.

Samples were processed for proteomic and metabolomic analysis. Proteomic normalization used the BCA assay and metabolic normalization used a dansylation assay [2]. Once processed, proteomic samples were analyzed using a Thermo Orbitrap Eclipse

Tribrid mass spectrometer, and metabolomics samples were analyzed using a Thermo Orbitrap QExactive Plus mass spectrometer. Data was analyzed using Proteome Discoverer 2.5, XGBoost, and Compound Discoverer 3.0.

Preliminary data shows separation between exposed and control samples for each OP-CWA. The proteomic analysis of VX confirms previously published *in vivo* data for the dysregulation of the TCA cycle [1]. Sarin and Soman also show significant dysregulation in the TCA cycle. Other preliminarily data indicates dysregulation for Sarin, Soman, and V1 in energy related pathways like glycolysis/gluconeogenesis and pentose phosphate pathway.

References

[1] Glaros, T. et al. (2020). Discovery of treatment for nerve agents targeting a new metabolic pathway. *Arch Toxicol 94*, 3249-3264. doi:10.1007/s00204-020-02820-4

[2] Gallagher, E. et al. (2023). Normalization of organ-on-a-chip samples for mass spectrometry based proteomics and metabolomics via dansylation-based assay. *Toxicol In Vitro 88*. doi:10.1016/j.tiv.2022.105540

Presentation: Poster

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An *ex vivo* mini-ovary provides a platform for studying ovarian biology, disease, toxicology

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The ovary consists of various staged follicles as its functional unit, which critically sustain female reproductive cycles and fertility. It is challenging to study the ovary due to the low throughput of whole animal models and limited biomass of premenopausal human ovaries. We have an established 3D hydrogel encapsulated *in vitro* follicle growth (eIVFG) system. Our previous studies demonstrated that eIVFG recapitulates key ovarian functions, including follicle maturation, hormone secretion, and ovulation. Herein, we investigated whether eIVFG preserves molecular signatures of these ovarian functions. Immature mouse follicles were cultured with follicle-stimulating hormone (FSH) for 8 days to stimulate follicle maturation, and grown mature follicles were treated with human chorionic gonadotropin hormone (hCG), an LH analog, for 14 hours to induce ovulation. Follicles were collected on day 0, 4, and 8 during the FSH window and at 0, 1, 4, and 8 hours post-hCG for single-follicle RNA-sequencing (RNA-seq) analysis. Multiple leveled RNA-seq analysis revealed that the majority of FSH- and LH-target genes and signaling pathways and their dynamics were well preserved in eIVFG. These results demonstrate that eIVFG faithfully preserves morphological, hormonal, and molecular signatures of gonadotropin-dependent ovarian functions. We next developed a closed vitrification system to cryopreserve immature mouse follicles. Vitrified follicles were thawed and cultured in eIVFG. The results showed that vitrified follicles had comparable reproductive outcomes to fresh follicles, and single-follicle RNA-seq analysis revealed comparable follicular cell transcriptome between fresh and vitrified follicles, indicating that vitrification enables a high-content follicle biobank. So far, we have used the eIVFG system to test 42 environmental chemicals and pharmaceutical compounds, including 10 bisphenol analogues, 7 flame retardants, 6 per-and polyfluoroalkyl substances (PFAS), 6 harmful algal bloom (HAB) toxins, and 8 pre-clinical compounds. Several chemicals exhibited ovarian disrupting effects. For example, AZD7762, a checkpoint kinase inhibitor, promoted follicle death by inducing granulosa cell apoptosis. Moreover, we were able to use eIVFG produce follicles exhibiting several key phenotypical and molecular changes in polycystic ovarian syndrome (PCOS), such arrested follicle maturation, anovulation, and hyper-androgen synthesis. Together, the eIVFG-based mini-ovary model is a powerful and high-throughput tool to study ovarian biology, disease, and toxicology.

Presentation: Poster

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Automated tool for renal biopsy diagnosis

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Introduction: Histological examination of the glomeruli in renal biopsies is essential for diagnosing many kidney diseases. However, manual identification and characterisation of glomeruli in biopsies requires trained histopathologists and can be a tedious, time-consuming task. Therefore, the development of automatic tools (e.g. AI) to help accelerate diagnostic workflows and improve detection accuracy is an unmet need. Such tools should have the ability to detect and count glomeruli of various sizes, shapes, and disease statuses across biopsy images and annotate these in the image. Advanced functionality could then be developed to classify glomeruli based on disease features (e.g., fibrosis, segmental sclerosis) and generate AI-assisted preliminary biopsy reports for review by the histopathologist.

Methods: Routine renal biopsies collected at Royal Free Hospital with patients' consent for research were used for this study

(n = 300). 2-5um biopsy sections were acquired as 8-bit RGB WSI using an Axio Scan Z.1 (20x/0.8NA). To ensure the general applicability of the model, multiple common histochemical stains were imaged and included in the training dataset. These digitised slides were then manually annotated by several histopathologists and nephrologists using QuPath to capture the features described in the matching to biopsy reports.

"You only look once" (YOLO) is a state-of-the-art, real-time object detection system that has previously been used to identify glomeruli in the PASM-stained section. For our work, we decided to adopt this approach using the latest version of YOLO that can function under multiple conditions (e.g., different stains and magnifications), and a Convolutional Neural Network model may then be used to classify various glomeruli diseases.

Results: Using the location information given by YOLO, the glomerular images could be cropped from the original image and tabularized alongside morphometric and histopathological readouts from our U-NET model that could segment the identified glomeruli from the background tissue and quantify features (e.g., % area of fibrosis).

Conclusion: This work shows that an automated image analysis pipeline can identify, quantify, and characterize glomeruli in 2D slides in seconds, with obvious utility for pathologists and clinicians. Further work to expand the functionality of this model and to validate it further in larger datasets is warranted.

Presentation: Poster

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Development of the human-relevant aerosol test platform HUMIMIC-InHALES for evaluating respiratory toxicity and systemic effects of inhaled aerosols

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A major limitation of current aerosol exposure systems is that they expose discrete subsets of human respiratory tract models to a fraction of a complex aerosol. This use of static *in vitro* cultures limits the power of the generated data to predict respiratory and systemic effects in humans.

Philip Morris International developed the Independent Holistic Air-Liquid interface Exposure System (InHALES) as a mechanical replica of the whole human respiratory tract. This aerosol delivery device perfectly matches the architecture and respiratory characteristics of the human respiratory tract and allows cultures to be exposed at the air-liquid interface in a model that includes three relevant respiratory tract compartments [1]. PMI engineered InHALES to implement TissUse's proprietary microphysiological HUMIMIC multi-organ-on-a-chip platform, which allows functional human tissue architecture homeostasis at a minute *in vitro* scale [2]. We further developed a novel HUMIMIC chip for plug-and-play insertion into InHALES to maintain and culture a human cell culture insert-based lung model with other tissue constructs or organ equivalents (e.g., the liver). The use of new materials significantly reduced the absorption within the chip and allowed for testing of hydrophobic compounds. We previously demonstrated the lung model's integrity and viability using CellTrace™ Calcein Red-Orange AM and CellTox™ Green staining. The airway cultures in the HUMIMIC chip are subsequently exposed to InHALES-generated physiological aerosols.

The HUMIMIC-InHALES system supports the development of any systemic assay for aerosol exposure, including acute and chronic toxicity and long-term treatment efficacy. Its combination of aerosol delivery testing with advanced cell culture systems provides physical mimicry of interconnected cell types that are "systemically" exposed to "inhaled" aerosols to assess the local effects of aerosols on: 1) biological barrier of the lung epithelia, 2) entry into blood circulation, and 3) eventual systemic effects.

References

[1] Steiner, S., Herve, P., Pak, C. et al. (2020). *Toxicol In Vitro 67*, 104909.

[2] Bauer, C. Wennberg, Huldt, K. P. et al. (2017). *Sci Rep 7*, 14620.

Presentation: Oral

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A surface-tension self-pumping microfluidic chip for suspension cell culture

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In this paper, we present a multiplexed microfluidic chip for suspension cell culture which allows for automated high-throughput suspension cell culture/imaging. The chip itself with the size of a microscopy slide contains 16 individual cell culture/imaging units, four chips can be arranged in a special chip holder the size of a microtiter plate, creating an array of 64 cell culture/imaging chambers. Each unit consists of a cell culture/imaging chamber with a volume of less than 5 µl each, an inlet and an outlet chamber, arranged in two rows with 9 mm spacing between the individual units. The lateral fluid flow is created by the different geometries (diameters) of inlet and outlet structures which creates a pressure differential between these ports due to a higher surface tension in

the smaller inlet port [1]. This allows the filling of the chamber, subsequent culture media flow and addition of staining dyes simply by adding a drop of liquid at the inlet port. This alleviates the need for external pumps and other flow control elements. As all functional fluidic elements are arranged according to a standardized 9 mm spacing, standard pipettors or manual multipettes are the only tools needed. The low flow speed results in minimal sheer stress in the cell culture and no culture perturbation during assay steps. As no external fluidic control elements are needed, there is minimal to no cell loss during fluid manipulation. The microfluidic chips are manufactured using injection molding of a cyclo-olefin polymer (COC or COP) with minimum background fluorescence, allowing for volume manufacturing. We have demonstrated several staining assays in this device with subsequent live cell imaging.

Reference

[1] Walker, G. M. and Beebe, D. J. (2002). *Lab Chip 2*, 131-134.

Presentation: Poster

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Comparative analysis of vascular transcriptomics in 2D, transwells, and organ-on-a-chip models

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It is well-established that cell culture conditions can modify phenotypic and genotypic signatures of endothelial cells. While vascular models are advancing in physiological complexity, there is a lack of validation studies directly comparing endothelial transcriptomics between 2D formats and various organ-on-a-chip or 3D microphysiological systems (MPS). This analysis could shed light on model decision-making by indicating changes in critical cell receptors and functional markers for specific vascular projects. The aim of the present study is to compare the expression of key endothelial cell markers across various culturing platforms: 2D cultures (traditional cell culture plates and transwells), 3-lane Mimetas chips, and in-house 3D-printed chips. While the 3-lane Mimetas and the in-house 3D-printed chips are considered 3D cultures, the former utilizes a bidirectional gravity-driven flow, and the latter uses a unidirectional perfusion pump-driven flow. To address our aim, we cultured Human Umbilical Vein Endothelial Cells (HUVECs) at their optimal cell densities, and a combination of gelatin and fibrin (gelbrin) was used as the extracellular matrix for the 3D cultures. RNA was harvested from the cells four days after reaching confluency in each condition and RNAseq analysis was performed. RNA expression data of the endothelial cell markers (tight junctions, maturation, inflammation, remodeling genes) in the 2D cell culture plate were compared with the transwells and 3D models. Ongoing analysis will determine the differential expression of these genes and validate the transition from 2D culture models to MPS for select endothelial cell markers. This cross-comparison study of 2D to 3D culture transcriptomics will provide the foundational data to support model development and guide optimal model selection for given project requirements.

Presentation: Poster

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Cocultivation of liver spheroids and human proximal tubule renal epithelium cells in a perfusable system

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Several microfluidic-based tissue culture systems are currently available, but many are optimized to cultivate a single cell type. Elucidating interplay between organs is essential to understand systemic responses to substances and their metabolism and elimination. A good example is interaction between the liver and kidneys: the liver transforms xenobiotic substances, which are then secreted by the kidneys. In addition, several drugs become nephrotoxic after bioactivation in the liver. For these reasons, there is increasing interest in developing *in vitro* microfluidic systems that reproduce liver-kidney interactions.

Here, we used a multicompartmental microphysiological system (Vitrofluid, Philip Morris International, Neuchâtel, Switzerland) to coculture liver spheroids and human proximal tubule renal epithelium cells (RPTECs). Primary RPTECs were cultured on collagen-coated transwells, while liver spheroids were generated with NoSpin™ HepaRG™ (Lonza, Basel, Switzerland) cells grown on agarose molds produced with the MicroTissues 3D Petri Dish system (Sigma-Aldrich, St. Louis, MO, USA). Cocultivation was initiated after RPTECs reached confluence and the liver spheroids were morphologically stable. Cocultures were maintained under static or perfused (flow rate of 150 µL/min) conditions. Cells were exposed to verapamil, and cell viability, albumin release, and cytochrome P450 activity were assessed. Gene expression levels of specific kidney and liver markers were also measured. To evaluate enhanced toxicity due to hepatic metabolism in cocultures, we quantified nephrotoxicity-associated miRNAs that were released in the supernatant.

Cocultures were viable and functional for 7 days under both static and dynamic conditions. The viabilities of both cell types

decreased after exposure to verapamil for 3 days as reflected by a reduction of released albumin and increased release of miR-29a, miR-34a, and miR-192. Liver spheroids were less sensitive to verapamil when perfused compared to the static condition. Our results clearly demonstrate successful implementation of the two-organ system for long-term coculture. Furthermore, the system is suitable for investigating liver-kidney interactions and evaluating nephrotoxic substances.

Presentation: Poster

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A modular barrier tissue platform with on-demand fluid flow and TEER measurement capabilities

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Tissue barriers separate organ and blood compartments in the body and feature selective transport properties across the interface. Biomimetic microphysiological tissue barriers have been developed to establish scalable disease models, including vascular dysfunction. The gold standard to assess barrier tightness is to measure the electrical resistance (i.e., the trans-epithelial/endothelial electrical resistance (TEER)) across a cell bi-layer. Most commercial TEER measurement tools (e.g., "chop stick" style electrodes) are designed to work with Transwell-type inserts. However, static Transwells cannot recreate the fluid flow conditions found *in vivo*.

To address this need, we have recently developed a modular platform that can be reconfigured to combine conventional (e.g., Transwell-like) open-well cell culture protocols with flow-enhanced microfluidic culture environments [1]. By employing a magnetically sealed flow module into this *m*odular *m*icrophysiological system enabled by a silicon *m*embrane (µSiM) platform [2], users can seed cells on either side of a transparent, 100-nm-thick nanoporous silicon membrane and apply dynamic flows that better represent the desired *in vivo* conditions. Here, we advance the platform to include a magnetically-latched TEER measurement lid that enables combined fluid flow and TEER measurements. As a preliminary demonstration, we highlight the experimental workflow that includes i) open-well cell seeding, ii) conversion to flow-enhanced culture with shear stimulation, iii) evolution of TEER values over time to assess barrier integrity, iv) and iv) molecular analysis of induced-pluripotent-stem-cell-derived brain microvascular endothelial cells (iPSC-BMECs) in the m-µSiM platform. We anticipate the ease-of-use and experimental flexibility of this platform will enable a variety of barrier tissue models to be developed.

References

[1] Mansouri, M. et al. (2022). The modular µSiM reconfigured: Integration of microfluidic capabilities to study in vitro barrier tissue models under flow. *Adv Healthc Mater*, e2200802. doi:10.1002/adhm.202200802

[2] McCloskey, M. C. et al. (2022). The modular µSiM: A mass produced, rapidly assembled, and reconfigurable platform for the study of barrier tissue models in vitro. *Adv Healthc Mater*, 2200804. doi:10.1002/adhm.202200804

Presentation: Poster

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Topologically constrained *in-vitro* networks express plasticity effects when stimulated electrically

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Our brains are capable of learning new information and retaining memories. However, the exact mechanisms by which the brain creates and retains new memories are not well understood. It is likely that neural plasticity plays a key role in these processes. We further believe that plasticity needs to be studied on a single cell level as opposed to a population level, as single neurons cannot easily access information at the population level. Plasticity research *in-vivo* with single cell resolution is feasible but encounters noteworthy limitations: It is difficult to record cellular activity of more than just a minuscule subset of neurons while still maintaining high temporal resolution in mammalian model organisms.

In order to record a considerable part of neuronal activity with single cell precision, we propose to reduce the complexity of a network. We do so by using primary neuronal networks cultured *in-vitro* containing in the order of hundreds of cells. Spiking activity can be recorded and induced through multi-electrode arrays (MEA). To further simplify the complexity of such networks, we physically confine them with the help of polydimethylsiloxane (PDMS) microstructures. Microstructures constrain the topology of a neuronal network by holding somata in place while simultaneously enforcing highly directed axonal growth [1].

We have recently shown that primary rat hippocampal neurons have stable spiking responses to electrical stimuli, which stay consistent for multiple hours [2]. At the same time, some networks displayed highly reproducible changes in spiking responses when stimulated repeatedly. These networks expressed modulations in

the spike timings based on how often a particular stimulus was applied in the recent past. Spike time changes are a form of plasticity. Approximately 20% of the spikes observed appeared at later time points (depression), while approximately 5% appeared at earlier time points (potentiation). While the number of depressing spike responses increased with culture age, no significant change was observed for potentiating effects. We believe that these results proof the usefulness of such *in-vitro* networks for plasticity research.

References

[1] Forró et al. (2018). *Biosens Bioelectron 122*, 75-87.
[2] Ihle et al. (2022). *Biosens Bioelectron 201*, 113896.

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Increasing predictability of antibody-triggered receptor mediated transcytosis and neurotoxicity of CAR-T based therapy with a novel blood brain barrier-on-chip model

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Blood brain barrier (BBB) models *in vitro* are crucial tools to aid in the pre-clinical evaluation and selection of BBB-permeant biotherapeutics. We developed a 3D human BBB-on-Chip co-culture model using the SynVivo microfluidic platform (SynBBB) and iPSC derived cells to model critical components of the BBB and blood-brain-tumor-barrier (BBTB). We established lumens under physiological *in vivo* shear stress conditions (5 dynes/cm^2) using iPSC derived or primary brain endothelial cells, while primary astrocytes and pericytes were cultured in the tissue channel. We deployed this BBB-on-Chip model to study antibody-triggered receptor mediated transcytosis by perfusing the endothelialized lumens with a well characterized single domain BBB-carrier FC5-Fc and non-crossing A20.1 control. We observed a significant increase in FC5-Fc transcytosis was observed under physiological shear stress conditions. Similar BBB crossing of FC5-Fc was observed in *in vivo* brain exposure experiments. We further deployed the SynBBB chips towards establishing a blood-brain-barrier tumor (BBTB) model in the pre-clinical assessment of glioblastoma-targeting EGFRvIII-CAR-T based immunotherapies. The BBTB model was able to discriminate cytotoxic efficacies of the different EGFR-CARs and provide a measure of potential alterations to BBB integrity. Collectively, these findings suggest that SynBBB technology can recapitulate the physiological characteristics of the BBB *in vivo* and offer a more predictive platform for assessing antibody transcytosis across the BBB and deciphering the mechanisms of CAR-T-induced BBB disruption, accompanying toxicity and effector function on post-barrier target cells.

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A hybrid silicon and polymer chip for 3D vascularized human beta-pancreatic model development

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Background: Research in diabetes needs relevant human model. The main challenge to develop three-dimensional model is to mimic pancreas microenvironment, particularly within the insulin-secreting unit. Langerhans islet is, indeed, highly vascularized, receiving 15% of the pancreatic blood supply (Rambøl et al., 2020). Consequently, neovascularisation is a major issue to maintain functional beta-pancreatic cells. *In vivo*, micro-vascularization is ensured by endothelial cells supported by other cell types forming a matrix allowing maturation of the network. The project aims at developing a human beta-pancreatic model on engineered chip to mimic the *in vivo* organ. The chip combines silicon technology and plastic polymer COC (Cyclic olefin copolymer). The vascularized pseudo-islet on chip would come entirely from human pancreatic cells and fed under medium flow.

Methods: A hybrid silicon and polymer COC chip was developed using silicon properties allowing good control of the chip geometry to optimize biology development. In parallel, heterotypic pseudo-islet secreting insulin was engineered from human beta-pancreatic, endothelial, and supportive cells to potentiate vascularization within the islet. Moreover, 3D neovascularization (human GFP-pancreatic endothelial and supporting cells) and network growth were performed in hydrogel of fibrin (natural polymer). Several cell ratios, medium and complementation were tested to ensure development of the microvascular network over time. Its morphology was evaluated throughout the study using fluorescent microscopy. The best conditions of micro-vascularization and pseudo-islet composition were selected and integrated on the chip described earlier. The three-dimensional vascularized pancreatic model was maintained under medium flow to mimic *in vivo* blood supply.

Results: Human pancreatic microvascular network was successfully integrated on chip and maintained beyond 15 days. Heterotypic pseudo-islets secreting insulin showed a compact spheroi-

dal structure with prevascularized core. Their transfer on chip is promising. Next step is to integrate pseudo-islet and the microenvironment on chip to reach anastomosis.

Conclusion: A fully human pseudo-islet secreting insulin in its microenvironment seems to be a relevant model to develop a new technology of organ on chip to optimize diabetes treatment. Silicon technology allows creating specific design to optimize the biology development and will allow integrating sensors to monitor different parameters on pseudo-organ as viability and functional capacity.

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A tumor-on-a-chip incorporating human-based hydrogels for easy assessment of tumor invasion and metastasis

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Metastatic progression of solid tumors is a dynamic and poorly understood process globally associated with high mortality. Unfortunately, the high failure rate of tumor drug discovery is steadily increasing owing to the limited predictability of the current preclinical models. *In vitro* tumor invasion modeling combining 3D biomimetic materials and multicellular spheroids has contributed with valuable insights to recreate the early mechanisms of tumor metastasis [1,2]. However, the integration of mechanical stimuli, tumor-stromal cell interaction, and tumor angiogenesis is still a challenge.

Leveraging the potential of organ-on-a-chip technology, a microphysiological osteosarcoma(OS)-on-a-chip model was developed in an attempt to recreate the early metastatic process of tumor invasion. In a fully human-based approach, a non-metastatic or metastatic OS tumor spheroid (MG-63 and 143B, respectively) was embedded in a platelet lysates-based hydrogel from human origin, and co-cultured with human bone marrow mesenchymal stem cells. Live imaging analysis revealed that the established model enables the recapitulation of the synergistic tumor-stromal cell and cell-extracellular matrix interaction of an invading tumor. Moreover, the increased invasiveness ability of metastatic cells compared with non-metastatic ones evidenced the feasibility of this model to reproduce this dynamic process. In fact, the migration of metastatic cells over long distances, reaching the outlet, demonstrates their aggressiveness. Such tumor proliferation and invasiveness ability demonstrated to be potentiated by the dynamic environment when compared with a static setting. The exposure of dynamic models to doxorubicin showed an improved drug resistance of the metastatic model, highlighting their suitability for drug discovery and prognosis. To further understand how dynamic environment influences tumor metastatic ability, protein and gene expression, as well as exometabolomic analysis was performed. Specifically, vascular endothelial growth factor was over-produced by 143B cells under dynamic conditions. Contrarily, MG-63 cells showed an increased expression of collagen, associated with a non-metastatic phenotype. Exometabolomics profiling of the developed OS-on-a-chip model further identified metabolic features correlated with tumor progression. Overall, the developed tumor model revealed a great potential to pursue mechanistic studies on early metastatic events, offering a fully human environment.

References

[1] Monteiro C. F. et al. (2020). *Adv Sci*.
[2] Monteiro C. F. et al. (2021). *Acta Biomater*.

Presentation: Poster

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High spatiotemporal resolution impedance mapping of Caco-2 epithelial barriers on CMOS-MEA chips

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Electrochemical impedance monitoring is a powerful label-free technique to continuously assess barrier properties of tissues in organ-on-chip platforms. Electrical cell substrate impedance sensing systems, typically using 1 or 10 electrodes with a diameter of ~250 µm (Bednarek, 2022), display improved resolution over Transwell-based ones. Increasing the spatiotemporal resolution in these systems requires smaller electrodes organized in denser arrays and integrated with a high-speed acquisition system. This has been realized though the development of complementary metal oxide semiconductor (CMOS) micro-electrode array (MEA) chips, which interface integrated circuits and multiplexers with high density small size electrodes (Lopez et al., 2018).

Here, we used CMOS-MEA chips with 16,384 subcellular-sized electrodes (Φ8 µm diameter, 15 µm pitch), to enable fast and single-cell resolution impedance mapping for Caco-2 cell barrier characterization. Using impedance spectroscopy, we showed that in these systems, the relative changes in impedance magnitude at 1 kHz can effectively describe attachment, spreading and barrier formation of Caco-2 cells.

Cell growth was monitored using |Z|1kHz measurement, showing an increase from 0.29 (± 0.04) MΩ to 1.25 (± 0.27) MΩ after 4 days, *in vitro*. Interestingly, the |Z|1kHz impedance mapping matches well with the optical imaging down to single cell resolution (membrane CellMask staining or anti ZO-1 antibodies). To prove spatial resolution in damage detection, the cell layer was selectively removed from specific electrodes. Only on the targeted electrodes a change in |Z|1kHz was detected, with values decreasing from 1.45 (± 0.08) MΩ to 0.39 (± 0.01) MΩ. Milder and temporary disruption of the barrier was induced by treating Caco-2 cells with 5 mM EGTA for 45 minutes, leading the |Z|1kHz value to decrease from 1.39 (± 0.16) MΩ to 0.80 (± 0.23) MΩ, indicating a milder effect on the impedance value.

Here, we showed that subcellular sized electrodes in high density arrays and integrated with CMOS circuits, allows for recording single cell information, fast and accurate mapping of barrier integrity. Therefore, it can be of great value in investigating epithelial/endothelial barriers. In future, Caco-2 co culture with Salmonella Typhimurium will be employed to prove the effectiveness of CMOS-MEA in monitoring pathological changes of barrier integrity.

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Lung on a chip with soft ECM tubular structures

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Compared to conventional 2D trans-well insert-based *in vitro* models, microfluidic lung-on-a-chip platforms have attracted substantial interest with advantages in recapitulating 3D micro-architecture, breathing movements, and accurate accommodation of multiple cell types. A wide range of airway/alveoli functional and disease models have been built on such platforms, such as chronic obstructive pulmonary disease (COPD), asthma, pulmonary edema, acute respiratory distress syndrome (ARDS), etc. [1]. Most of these platforms so far rely on lithography-fabricated parallel microfluidic channels separated by a synthetic membrane. While air liquid interface (ALI) culture is established, they are limited in short culture time with minute non-uniform shear stress [2]. Specially, regarding ventilation-induced lung injury (VILI), "atelectrauma" was successfully recapitulated for epithelium injury study during the lung recruitment process but limited to "meniscus-formation" induced collapse and reopening in such platforms, regardless of compliance and substrate ligand [3]. Here, an ECM-based and 3D-printed tubular structure was selected to recapitulate the airway microenvironment. Combined with a custom-designed thermoplastic tube hosting device and airflow system, confluent human small airway epithelium was developed and further matured during long-term ALI culture. The collapsible, stretchable, and perfusable nature of these soft collagen tubular structures allows accurate recapitulating physiological airway movement including shear stress, cyclic stretching, and repetitive compliant collapse & reopening. The relation between transmural pressure, air finger propagating, and the effect of surfactant and flow resistance during ventilation were studied. Eventually, airway epithelium injury during repetitive "compliant" collapse and reopening was analyzed under varied conditions. We anticipate this approach to enable a deep understanding of ventilation-relevant lung disease and have broad applications in other ECM-based organs-on-chip.

References

[1] Slutsky, A. S. and Hudson, L. D. (2006). *N Engl J Med 354*, 1839.

[2] Huh, D., Fujioka, H., Tung, Y. C. et al. (2007). *Proc Natl Acad Sci U S A 104*, 18886.

[3] Tavana, H., Huh, D., Grotberg, J. B. et al. (2009). *Lab Med 40*, 203.

Presentation: Poster

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Design and fabrication of a piezoresistive microcantilever strain sensor for measurement of contractile muscle force generation

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Microcantilever sensors are highly sensitive tools used in a wide variety of applications to measure small scale mechanical movement and are commonly used in biomedical applications. In biomedical microelectromechanical systems (bioMEMS), cantilevers are commonly used for the measurement of contractile force of muscle tissues using *in vitro* microphysiological systems. In existing microcantilever sensors in body-on-a-chip systems, the cantilevers' readout often relies on bending and resultant surface stress that requires imaging methods or optical laser deflection of cantilevers for calculating the contractile force of human cardiac and skeletal muscle tissues since they cannot acquire a direct electrical signal from the cantilever. This work describes the design, fabrication, and characterization of a bioMEMS piezoresistive microcantilever strain sensor and electronics for the continuous force measurements for *in vitro* cardiac systems. The device is a silicon can-

tilever with integrated thin film gold sensing material. This design has been driven by mathematical modeling to achieve the highest effective gauge factor and with balanced stress of the cantilevers. A serpentine pattern has been used to maximize the contribution of the sensing element and travels the length of the cantilever to have the ability to capture cardiac contraction along the length of the beam. This microcantilever sensor will enable high-throughput, real-time, continuous force measurements for *in vitro* cardiac muscle systems and for multi-organ body-on-a-chip systems. These systems will prove invaluable for basic physiological investigation, pharmaceutical compound development, toxin detection, personalized medicine, and predictive toxicology.

Presentation: Poster

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Addressing genetic backgrounds and shared phenotypes in autism spectrum disorder

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Autism spectrum disorders (ASD) are a major public health concern worldwide, affecting up to 1 in 44 children in the US, however the etiology of this complex disorder remains unclear. There have been many genetic factors identified that contribute towards ASD risk; however, this alone does not explain the increase in prevalence, nor the clinical heterogeneity across similar mutations. Environmental factors including pesticides and air pollution have been linked to ASD, yet the cellular and molecular mechanisms are unknown. Additionally, there is no effective animal model for ASD, further complicating research on this complex disorder.

To study this, we employ a brain organoid microphysiological system to better understand the potential convergent pathways of ASD genetic predispositions, and their interplay with environmental exposures. We hypothesize that different genetic risk factors will have unique and shared phenotypes. We additionally hypothesize that individuals with genetic predispositions to autism are more sensitive to environmental exposures, and the interplay of genetics and exposure triggers the manifestation of disease. Previous work in our lab has utilized an IPSC derived, brain organoid model to demonstrate increased susceptibility to acute, high dose, chlorpyrifos exposure (an ASD associated pesticide) in brain organoids harboring a CRISPR induced heterozygous CHD8 deletion. CHD8, chromodomain helicase binding domain 8, is a genetic risk factor for autism. Currently we are utilizing a low dose, chronic exposure model, and demonstrated that brain organoids with CHD8 deletion respond in unique ways to chlorpyrifos exposure. For example: in unmutated organoids, chlorpyrifos leads to higher expression of CHD8, yet in brain organoids with heterozygous Chd8 deletion, exposure results in significantly lower CHD8 expression.

We are also working to expand this model into novel genetic backgrounds, by generating brain organoids from iPSCs with 16p11.2 mutations from patients with varying ASD severity, as well as patients with idiopathic autism, with the goal of completing a low-throughput gene and environment screen. This work will improve our understanding of the developmental insults and pathophysiology of ASD, establish brain MPS as a key tool for studying autism pathogenesis, and further elucidate the molecular mechanisms behind gene and environment interactions contributing to the disease.

Presentation: Poster

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Introduction of circulating factors to the µSiM-BBB for *in vitro* studies of sepsis-associated brain injury

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Sepsis is a maladaptive response to infection with a 25-50% mortality rate and a high incidence of cognitive impairment among sepsis survivors [1]. Given that sepsis originates from outside the brain, it is believed that the causative factors must traverse the bloodstream and penetrate/act on the blood-brain barrier (BBB) to induce brain damage. Directly examining the brain of septic patients is unfeasible, and animal models of sepsis do not accurately reflect the human conditions [2]. Therefore, an *in vitro* model of the human BBB, incorporating the flow of potentially harmful factors, is crucial in investigating sepsis-associated brain injury.

We are utilizing the recently established modular µSiM platform [3] (microphysiological system enabled by a silicon nanomembrane) to create models of the BBB and neurovascular unit (NVU) and evaluate the underlying mechanisms of brain injury during sepsis. The ultrathin (100 nm), high permeability, and glass-like optical properties of the nanomembranes used in the µSiM enables unhindered crosstalk of soluble factors between blood and brain compartments, and live-cell and label-free imaging of dynamic changes in the BBB. We have developed a suite of assays for the µSiM-BBB, including *in situ* and end-point assessments of permeability [3], and automated analysis of leukocyte transmigration from the blood to the brain side of the chip.

In this report, we will highlight the advancements to the µSiM-BBB that enable the introduction of circulating factors into the blood side while simultaneously monitoring the effects on barrier function and neuroinflammation. The circulating factors being studied include "cytomix" (a septic-like cocktail of TNFα, IFNγ, and IL1-β), glycocalyx degradation agents, immune cells, DAMPs, and cell-free hemoglobin. Our preliminary data show rapid BBB breakdown and astrocyte activation (as a measure of

astroglial neuroinflammation) within hours of the introduction of cytomix [3], as well as more prominent transmigration of leukocytes once neuroinflammation established on the brain side of the chip. These results suggest that the cytokine storm may initiate a self-reinforcing cascade of neuroinflammatory events leading to injury, rather than directly causing the injury itself.

References
[1] *JAMA 315*, 801-810 (2016).
[2] *PNAS 110*, 3507-3512 (2013).
[3] *Adv Healthc Mater 11*, 2200804 (2022).

Presentation: Poster

517

Integrated microfluidic platform for tumor spheroid-induced 3D angiogenesis model

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Research on anti-cancer drug development have been progressed but insufficient reliability of animal experiment due to biological differences, the clinical trial process shows high failure rate. To overcome this limitation, polydimethylsiloxane (PDMS) based 3D *in vitro* models have been developed to mimic the microenvironment of human body. However, due to the characteristics of PDMS, it has low efficiency for manufacturing and is inadequate to use in the preclinical process of the drug. High-throughput microfluidic platforms fabricated by plastic-based injection molding have been developed to solve material limitations, but additional processes were required to handling spheroids. Recently, we developed 3D tissue culture platform that integrates spheroid formation process and cellular microenvironment reconstruction process (All-in-One-IMPACT). In this study, we performed a 3D tumor-induced angiogenesis in the developed platform. We analyzed the angiogenesis tendency varying with hydrogel concentrations and types of cancer cell. Also, we treated anti-cancer drugs to the patient-derived cancer cells (PDCs) tumor spheroid angiogenesis model and observe the effects on angiogenesis according to the concentration of the drug. We demonstrated that our platform enables to study of the tumor microenvironment (TME) and drug screening. We expect the platform contributes to further research on complex mechanisms of TME and predictive preclinical models.

Presentation: Poster

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Implementation of a human cell-based malaria-on-a-chip phenotypic disease model for drug efficacy evaluation

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Of all the Plasmodium spp. found in humans, the infectious protozoans responsible for malaria, the falciparum species is the largest contributor to malaria mortality rates. The need for a human-based platform to model disease pathophysiology and monitor drug efficacy is much needed for preclinical drug development. The previously established Malaria-on-a-Chip model, which has demonstrated the capability of culturing all stages of the parasite erythrocytic lifecycle, was utilized to further evaluate drug efficacy and off-target toxicity of common antimalarial therapeutics. The optimum efficacious doses for chloroquine, artesunate, and lumefantrine were determined by analyzing the max tolerable dose and no observed adverse effects level in this functional, human, multiorgan, serum-free platform. These doses were then implemented to study the clearance times of *P. falciparum* and any potential recrudescence of the chloroquine sensitive and chloroquine resistant strains, 3D7 and W2. Furthermore, this study aims to identify specific targets of the parasite erythrocytic lifecycle for each compound using flow cytometry. Additionally, the viability of all organ constructs, as well as hepatic metabolic activity and splenic immune response, were monitored over the course of infection and treatment. Compounds were delivered as a monotherapy in a single, bolus dose immediately after infection with the parasite. Following administration of treatment, a dose dependent clearance of the parasite was observed in both strains for all compounds. Recrudescence of the 3D7 strain was observed by day 7 for all chloroquine and lumefantrine treatments while recrudescence was not observed by day 7 with artesunate treatment. Recrudescence was not observed in the W2 strain. However, W2 infected systems exhibited a stabilization of parasitemia levels by day 7 when treated with chloroquine and lumefantrine, while those treated with artesunate continued to diminish. A significant dose dependent decrease in infected organ viability resulted from chloroquine treatment but no significant decrease in infected organ construct viability was observed when treated with lumefantrine or artesunate.

Presentation: Poster

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Functional neuronal platform to investigate glial-neuron contributions towards AD in a hiPSC derived triculture system

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation or impaired clearance of amyloid-β (Aβ), hyperphosphorylated Tau (p-Tau), as well as aberrant inflammatory response by glial cells. Many years of research have been dedicated to understanding AD pathogenesis with regards to Aβ and Tau, with little regard to the contribution that glial cells exert over the course of AD progression. Recently, *in vitro* studies have developed a central nervous system triculture model consisting of neurons, astrocytes, and microglia in an attempt to recapitulate physiological human brain-like conditions for the study of AD and other neurological disorders. In this study, this avenue was explored through the integration of these cell types derived from human induced pluripotent stem cells (hiPSCs) with human-on-a-chip (HOAC) technologies. Here, the focus was on characterizing the modulatory effects that glial cells have on the molecular and electrophysiological functions of hiPSC-cortical neurons that carry the familial AD (fAD) mutation in $PSEN1^{A246E}$. Immunocytochemical (ICC) staining was able to detect the presence of biomarkers associated with AD pathology, such as p-Tau and Aβ, as well as confirm the presence of the three cell types in one system. A variety of pro- and anti-inflammatory cytokines secreted by these cells were detected by a sandwich membrane immunoblot assay, furthering our understanding of the inflammatory contributions of glial cells in AD. Additionally, the action potential characteristics of neurons in monoculture and triculture conditions were observed and compared through whole-cell patch clamp electrophysiology. A hiPSC-CN microelectrode array (MEA) model capable of generating functional long-term potentiation (LTP) has been established and validated [1], which lends itself as a platform for the observation of the basic electrical activity of neural networks as well as LTP induction and maintenance – now with the addition of glial cells. This AD triculture HOAC model furthers the understanding of glial contributions in AD pathology, and can be applied towards the screening of current and novel treatments for AD.

Reference

[1] Autar, K. et al. (2022). *Stem Cell Rep 17*, 96-109.

Presentation: Poster

521

Human neuron-on-a-chip platform to automate the screening of compounds targeting Alzheimer's disease

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Neurological disorders affect approximately 20% of the world population and are among the top ten leading causes of disability and death. There is no cure and no efficient therapy for the vast majority of neurological disorders and 99.6% of the drugs targeting neurological diseases that pass animal tests fail on human trials. Animal experiments are currently the gold standard to test toxicity and efficacy of compounds. However, it is not feasible to test all compounds in the market with current guidelines due to high costs; long testing times, high number of animals required and low reproducibility [1]. New, human based, and more predictive models are required to accelerate the development of effective therapies. At Ananda Devices, we have developed technology for rapid growth and precise organization of human neuronal networks-on-a-chip [2,3]. We adapted the technology to multi-well microplates (NeuroHTS™) and combined with analysis software to automate the analysis of 7+ parameters on neuronal morphology, neurite growth, synapse formation and network dynamics in 3000 individual neurons per plate. Here, we used this platform to compare neuronal morphology, network dynamics, synapses and electrical function of neurons derived from iPSC cells from Healthy and Alzheimer's Disease donors. Using this high-definition analysis, we were able to identify key differences in axonal growth, axonal thickness, neuronal connections, synapse formation, synaptic maturity, tau distribution in cells derived from Alzheimer's Disease donors and Healthy donors. The results were validated using RNAseq analysis highlighting key signaling pathways involved in neurodegeneration. The high sensitivity of the NeuroHTS™ platform enables rapid and automated compound screening directly on patient's derived cells. The main advantages of the technology are faster acquisition of neuronal data and generation of more predictive data of compounds' safety and efficacy prior to exposure to humans.

References

[1] Cook et al. (2014). *Nat Rev Drug Discov*.
[2] Magdesian et al. (2016). *Biophys J*.
[3] Magdesian et al. (2017). *J Vis Exp*.

Presentation: Poster

522

Industry standards to guide mass spectrometry spatial techniques for PKPD modeling for organ-on-a-chip research

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The coupling capabilities of Mass Spectrometry provide a pathway to expand and improve the ability to chemically characterize the Pharmacokinetic (PK) parameters as an indirect correlation to any of the measured Pharmacodynamic (PD) parameters by following a cross-validation workflow. Drug Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) information can be obtained by using Mass Spectrometry Imaging (MSI) applications, which generate chemically specific and spatially resolved ion distribution profiles for given compounds and metabolites. MSI-based pharmacokinetic imaging analysis enhances the knowledge of the spatial PKPD parameters for compounds, providing a histological framework concerning the drug distribution and metabolic pathways within a cellular system. One approach as Spruill et al. outlines is to acquire spatial analysis of the "tissue section" using MALDI as an ionization source for MSI but redesigned using the housing material from the organ-on-a-chip device. Using this as a reference point, the microfluidic device would be treated as tissue and analyzed accordingly for drug spatial distribution. For clinical applications, the use of parameters derived from the literature would undoubtedly reduce the need for extensive experimentation. Through the implementation of stringent Quality Control Standards within the laboratory that manufactures the device at all stages of the process, it is possible to evaluate the potential viability of moving forward with a drug candidate in human trials without the need to generate unnecessary animal models as part of a risk assessment. For this technology to be viewed as a viable alternative to animal testing, it would be highly advantageous to adopt a methodology, supported by the FDA – considering recent trends in the agency to support an initiative to investigate the feasibility of integrating human-on-a-chip devices within a clinical setting. Developing a testing regiment based on this framework would overcome any skepticism from the agency in the long run.

Presentation: Poster

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Analyzing label free leukocyte trafficking dynamics on a microvascular mimetic with computer vision techniques

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Microphysiological systems (MPS) are increasingly being used to model the human immune system, including trafficking of immune cells from vessels to surrounding tissues. While typically utilized in endpoint studies, such as ELISA, MPS featuring ultrathin, nanoporous silicon nitride membranes (μSiM devices) also enable high magnification phase contrast microscopy recordings [1]. Notably, the imaging plane can be set directly at the endothelial interface in a μSiM device, resulting in high-resolution capture of an endothelial cell (EC) and leukocyte coculture reacting to varying stimulatory conditions. Data generated from recording observations can then be used to elucidate disease mechanisms related to vascular barrier dysfunction, such as sepsis. Leukocyte morphology in these recordings is dynamic, however, as leukocytes manifest differently depending on their location with respect to the endothelium (luminal or abluminal). Consequently, labor-intensive manual analysis is required as conventional image processing techniques are incapable of extracting the spatiotemporal profiles and bulk statistics of numerous leukocytes responding to a disease state. In order to overcome this limitation, we describe a machine learning (ML) pipeline that uses a semantic segmentation algorithm and convolutional neural network classification script that, in combination, is capable of automated and label-free leukocyte trafficking analysis in a coculture mimetic of the vascular wall. The developed computational toolset has parity with manually tabulated datasets when characterizing leukocyte spatiotemporal behavior, is computationally efficient, and capable of managing large imaging datasets in a semi-automated manner. Following validation, we further demonstrate differential leukocyte transmigratory activity in response to a simulated cytokine storm ("cytomix") using models of the microvasculature featuring primary human cells or iPSC-derived brain microvascular cells. Interestingly, both models show that abluminal stimulation with cytomix produces a more robust transmigration response versus luminal stimulation. Thus, the combination of live cell imaging and ML for image analysis is revealing potential differences in the microvascular response to an inflammatory challenge originating in the "tissue" side of the μSiM device.

Reference

[1] Salminen, A. T., Tithof, J., Izhiman, Y. et al. (2020). Endothelial cell apicobasal polarity coordinates distinct responses to lu-

minally versus abluminally delivered TNF-α in a microvascular mimetic. *Integr Biol (Camb) 12*, 275-289.

Presentation: Oral

524

Cross-species biomarker identification for drug induced vascular injury using a high-throughput organ-on-chip platform

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Drug-induced vascular injury (DIVI) is an adverse event that arises during preclinical testing and contributes to the termination of promising drug candidates. Currently, DIVI is diagnosed via histopathology which is prohibitively invasive and precludes real-time monitoring. Additionally, numerous drugs cause DIVI in animals but whether DIVI occurs in humans remains controversial. Thus, there is significant pharmaceutical and regulatory interest in identifying sensitive, translatable biomarkers associated with DIVI across species.

Our objective was to develop physiologically relevant human and rat models of the vasculature in our high throughput organ-on-chip platform to characterize potential DIVI-associated biomarkers using tool compounds.

Rat or human co-culture models (endothelial and vascular smooth muscle cells) were seeded in microfluidic devices that were exposed to feeder flow (0.1 dyn/cm^2) or physiologically relevant fluid shear stress (7 dyn/cm^2). After model stabilization, Fenoldopam, Dopamine, or Minoxidil (0, 50, 100, 200 μM) was administered daily over the course of 4 days. Supernatants were collected to assess cytotoxicity (LDH assay), secreted factors (Luminex), and circulating microRNAs (qPCR). On day 5, samples were fixed for immunofluorescence staining to assess cell morphology. Single cell RNA-sequencing studies are ongoing.

We confirmed injury by immunostaining (endothelial tight junction disruption) and identified several potential translational biomarkers of DIVI, such as CXCL1, VEGF, and microRNAs, including similarities and differences across species. This study highlights the utility of high-throughput organ-on-chip platforms to rapidly and effectively model complex injury states, facilitating biomarker discovery and validation for improved preclinical studies and eventual clinical implementation.

Presentation: Poster

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Investigation of the efficacy and off-target toxicity of both acute and chronic opioid overdose and naloxone recovery in multiorgan human-on-a-chip systems

Stephanie Lang[1], Aakash Patel[1], Suruchi Poddar[1], Hao Wang[1], Daniel Nierenberg[1], Marcella Grillo[2], Jeffrey Roles[2], Jules Klion[2], Christopher Long[2], Xiufang Guo[1] and James Hickman[1]

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Millions of Americans each year report having chronic pain which is commonly treated by physicians with opioids. The highly addictive properties of opioids have led to their widespread misuse, which contributes to the opioid epidemic. The number of fatal overdoses has increased from 70,360 in 2019 to 93,331 in 2020, which is in large part due to the isolation and delay of primary care caused by the COVID-19 pandemic (Centers for Disease Control and Prevention, 2022). In this study, we aim to investigate acute and chronic treatment of naloxone as a method for reversing methadone induced overdose in a multi-organ microphysiological system (MPS) comprised of human derived preBötzinger neurons, cardiac, skeletal, and liver cells. This interconnected pumpless system has continuous recirculation, allowing for crosstalk between each cell type. Respiratory depression associated with opioid overdose has been shown to be caused by the activation of μ-opioid receptors in the PreBötzinger complex which leads to inhibition of neuron activity. PreBötzinger neurons and cardiomyocytes were cultured on microelectrode arrays (MEAs) to measure the electrophysiological properties of the neurons and the conduction velocity of cardiomyocytes. Skeletal muscle and cardiomyocytes were cultured on cantilevers to investigate the contractility and amplitude. Additionally, drug metabolism and clearance were determined by analyzing the fluid samples from the multi-organ system with high-performance liquid chromatography. Creating this well-defined system supplements animal experiments which not only poses an ethical concern, but are time consuming, costly, and can be unreliable in predicting drug efficacy and toxicity in humans. The incorporation of multiple cell types in this interconnected system can more accurately recapitulating human physiology and allowing for cellular communication across all cell types. Furthermore, this MPS and many other emerging MPS technologies have the capability of being used as platforms for evaluating current and novel treatments on any diseased condition – eventually streamlining and expediting the drug development process.

Center for Disease Control and Prevention, 2022. A qualitative assessment of circumstances surrounding drug overdose deaths during early stages of the COVID-19 pandemic. https://www.cdc.gov/drugoverdose/databriefs/sudors-2.html

Presentation: Poster

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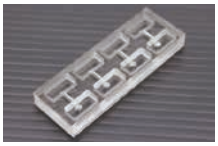
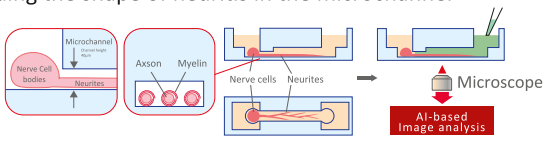


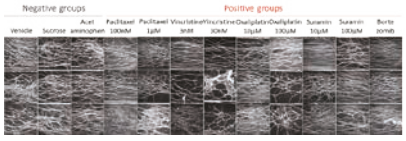
Collaboration with Prof. Ikuro Suzuki

TOHOKU INSTITUTE OF TECHNOLOGY

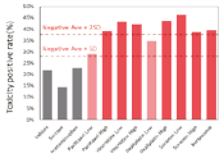
Peripheral Neurotoxicity Evaluation based on AI image analysis reading the shape of neuritis in the microchannel



- Representative local immunofluorescence image samples of neuntes in a microchannel after drug administration.



- AI showed the potential for drug-induced neurotoxicity even at low concentrations.



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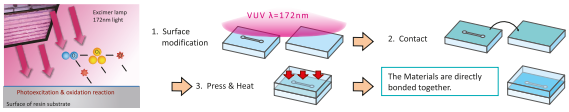

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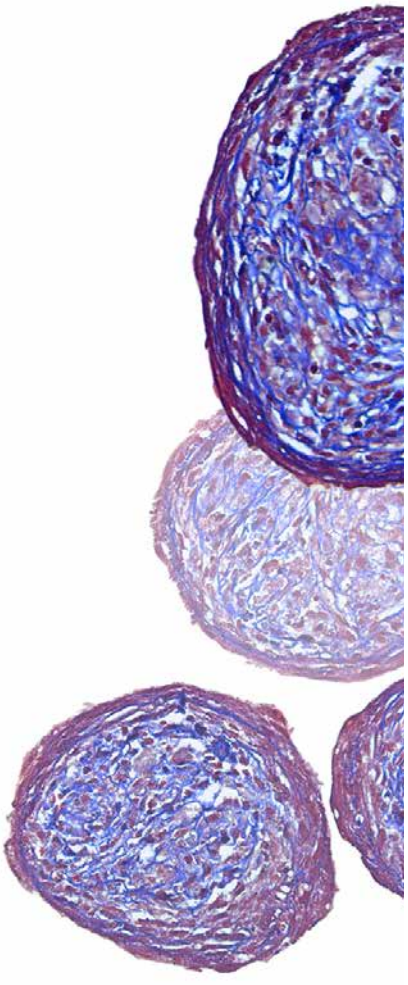

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Application of flow cytometry for *Plasmodium falciparum* quantification for a malaria-on-a-chip model

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Malaria caused by the parasite *P. falciparum* continues to be a leading cause of mortality worldwide. Quantification of parasitemia throughout a treatment regimen is performed to ensure effectiveness of the antimalarial course chosen. Classically this measurement is calculated through use of a Giemsa staining protocol, a manual process in which blood is smeared and stained on a slide to observe infected erythrocytes through microscopy. While it is known as the gold standard of malarial parasite quantification, this technique is time consuming and labor intensive, and requires counting of hundreds of red blood cells to acquire a reliable output. Results are also prone to subjectivity, as the staining and counting methodology may vary between researchers. Given these shortcomings, an objective technique would benefit in establishing a more uniform quantification method. For this study, an automated technique utilizing flow cytometry to detect intraerythrocytic parasite growth was adopted and optimized [1]. *P. falciparum* infected blood flasks were maintained for 1 month, then samples were collected every other day for immediate staining and processing on the cytometer. Samples were stained with SYBR Green I nucleic acid gel stain and plotted on FITC histograms for analysis. The results of this analysis were validated by comparison to traditional Giemsa stain counts. This method allowed for efficient and robust parasitemia quantification while also eliminating observer variability. In addition to quantifying parasitemia this assay had other valuable applications, as it was used to observe the effects of the antimalarials chloroquine, artesunate, and lumefantrine on specific life cycle stages of the parasite. This enhanced our understanding of the pathophysiology of the malaria parasite in our system and may also be advantageous in informing effects of novel therapeutics in the future.

Reference

[1] Izumiyama, S., Omura, M., Takasaki, T. et al. (2009). Plasmodium falciparum: development and validation of a measure of intraerythrocytic growth using SYBR Green I in a flow cytometer. *Exp Parasitol 121*, 144-150. doi:10.1016/j.exppara.2008.10.008

Presentation: Poster

527

Flow-induced ACE2 expression allows for SARS-CoV-2 infection of endothelial cells in a vascularized microphysiological system

Christopher Hatch[1], Sebastian Piombo[1], Jennifer Fang[2], Johannes Gach[1], Makena Ewald[1], William van Trigt[1], Don Forthal[1] and Christopher Hughes[1]

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/COVID-19) has caused over 7 million deaths worldwide since first being identified. High mortality COVID-19 is associated with a hyperaggressive inflammatory response characterized by hypercytokinemia ("cytokine storm") and endothelial damage. We have developed a microphysiological system (MPS) disease model based on our previously reported Vascularized Micro Organ (VMO) platform [1,2] that recapitulates COVID-19 hypercytokinemia, and have used this to investigate the relevant molecular pathways. Firstly, we find that ACE-2, the cellular receptor for SARS-CoV-2, is upregulated on endothelial cells (EC) by physiological shear and that viral infection of EC is enhanced under flow conditions in the VMO compared to monolayer cultures. Exposure of the VMO vasculature to SARS-CoV-2 pseudovirus reduces ACE2 expression while increasing expression of ICAM-1 and VCAM – two key pro-inflammatory adhesion molecules on EC. These results are potentiated by addition of angiotensin-2, which is normally rapidly degraded by ACE2. In addition, soluble inflammatory molecules, including the cytokines IL-1 and IL-6, are induced by viral infection, along with Factor VIII, a pro-coagulatory molecule. Importantly, soluble recombinant ACE2 was able to block the proinflammatory effects of pseudo-typed virus, whereas the TMPRSS2 inhibitor camostat had only modest effects. Finally, addition of supernatant from infected VMOs to fresh EC led to NFκB activation and enhanced cytokine production, demonstrating the possibility of localized infection having downstream, systemic effects. In summary, we have created an MPS platform that recapitulates the hypercytokinemia seen in many COVID patients and shown that this can be triggered by vascular activation subsequent to virally-mediated loss of ACE2 from the EC surface. Moreover, viral infection also triggers release of Factor VIII, which has the potential to enhance the vascular thrombosis known to be associated with long-COVID.

References

[1] Moya, M. L., Hsu, Y. H., Lee, A. P. et al. (2013). In vitro perfused human capillaries. *Tissue Eng 19*, 730-737.

[2] Phan, D. T., Wang, X., Craver, B. M. et al. (2017). A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. *Lab Chip 17*, 511-520.

Presentation: Poster

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Vascular malformations in a novel HHT-on-a-chip microphysiological system model

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In Hereditary Hemorrhagic Telangiectasia (HHT), mutations in the endothelial-expressed genes ACVRL1 (Alk1) and ENG (Endoglin) drive vascular malformations (VM) that include arteriovenous malformations (AVMs) and telangiectasias, which are comprised of overgrown small vessels. Current efforts to identify HHT treatments are challenged by lack of available *in vitro* models that completely mimic the intact microenvironment in which healthy and diseased blood vessels form. Here, we present a novel HHT-on-a-chip microphysiological system (MPS) model wherein primary human endothelial cells (EC) self-organize into a perfused blood vessel network that connects with EC-lined outer channels representing a high-flow artery and a low flow vein. Network formation is supported by fibroblasts and pericytes. Using this platform, we show that primary human EC engineered (via RNA silencing) to lack ACVRL1 (Alk1) expression form aberrant blood vessel networks under low flow/shear conditions reminiscent of telangiectasias. At higher flow levels, with concomitant higher vessel wall shear, arteriovenous shunts typical of HHT are generated. Using a mixture of wild-type and knockdown cells, expressing different fluorescent markers, we find that arteriovenous shunts are mosaic structures comprised of both Alk1-intact and Alk1-deficient EC, and that Alk1 is protective against malformations in growth-activated vessels. Single-cell RNA sequencing of lesions reveals strong up-regulation of angiogenesis-associated genes in Alk1 knockdown cells. Consistent with this, vascular malformations are prevented by the anti-angiogenic drug pazopanib, which targets VEGFR2, in line with two recent clinical studies [1,2]. Taken together, we describe a robust and scalable MPS disease model of HHT that will enable further studies into the pathophysiology of the disease as well as drug discovery and testing.

References

[1] Faughnan, M. E., Gossage, J. R., Chakinala, M. M. et al. (2019). Pazopanib may reduce bleeding in hereditary hemorrhagic telangiectasia. *Angiogenesis 22*, 145-155.

[2] Parambil, J. G., Gossage, J. R., McCrae, K. R. et al. (2022). Pazopanib for severe bleeding and transfusion-dependent anemia in hereditary hemorrhagic telangiectasia. *Angiogenesis 25*, 87-97.

Presentation: Oral

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An MPS model to study the effect of infinitesimally small shear stress on the morphology and performance of a hybrid proximal tubule microtissue

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The renal proximal tubule is the major site for reabsorption of molecules from the glomerular filtrate. Despite its small size it plays a major role in homeostasis. Prior *in vitro* models of the organ have used immortalized or primary cells, exclusively (Lin et al., 2019). Here, for the first time, we employ a combination of hiPSC-based pseudo-proximal tubule cells and immortalized cells (RPTEC/TERT1) to model the organ function. Such a coculture resulted in anomalous upregulation of certain transporters at mRNA and protein levels, compared to either cell source, leading to improved filtration efficiency. While the reason of such synergy is under investigation, we show that a miniscule amount of shear pressure exerted on the apical side causes discernable morphological improvements on this hybrid epithelial microtissue.

Organ models using iPSC-based cells are expected to offer superior performance owing to their *in vivo* like characteristics. However, it was established that upon 2D culture the expression levels of proximal tubule markers drop as they appear to lose their stem cell niche. Interestingly, we found that once the iPSC-derived cells are cocultured with RPTEC/TERT1 cells in our MPS, these markers are revived. Enhancements in the protein and functional levels were confirmed by immunostaining and uptake/filtration assays, respectively, for MDR1 and SGLT2.

Application of a small-amplitude flow-induced shear stress (0.06 dyn cm^{-2}) on the apical side, for two days past confluency, caused tangible increases in the density and length of microvilli. Such shear pressure is even lower than the minimum estimated values *in vivo* (Duan et al., 2008). In addition, cells obtained a more columnar format under shear stress, resembling the *in vivo* conditions, with the improvement being more pronounced in the coculture tissue. TEM observations showed formation of denser tight junctions under shear stress. We modified our MPS to enable online recording of the transepithelial electric resistance (TEER). Measurements showed that the post-confluency sheet resistance of the coculture saturates at a higher value that that of the RPTEC/TERT1 tissue. This study clearly shows the synergy between iPSC-derived and immortalized proximal tubule cells and how shear stress promotes maturation of the hybrid tissue.

Presentation: Poster

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Recapitulating acute myeloid leukemia (AML) phenotypes *in vitro* using a 3D model of the bone marrow microenvironment (BMME)

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Acute myeloid leukemia (AML) is the most aggressive type of adult acute leukemia. Mortality and morbidity are high and associated with the loss of normal hematopoiesis due to the disruption of the bone marrow (BM) microenvironment (BMME). We showed that leukemic cells express high levels of the chemokine CCL3 that inhibits osteoblastic cell function and is critical for the maintenance of leukemogenesis in a murine model (Ackun-Farmmer et al., 2021; Staversky et al., 2018). However, there are currently no reliable *in vitro* models that can fully recapitulate the BMME during AML. To bridge this gap, we aim to develop human and mouse AML-BMME-on-a-chip (AML-chip) to study the leukemic cell-BMME interactions *in vitro*. Previously, we have published a healthy mouse BMME model (BMME-chip) containing osteoblastic and endothelial components using Chip S-1® (Emulate™) that can culture hematopoietic stem and progenitor cells (HSPC) and maintain their long-term engraftment function (Sharipol et al., 2022). To develop the AML-chip, we cultured leukemic cells isolated from a murine AML model in the fibrin gel-hematopoietic component of the BMME-chip with HSPCs, BM stromal cells (BMSCs), and osteoblasts. After 14 days, we found that leukemic cells comprised 29.00 ± 2.35% of total live cells equivalent to leukemic cell burden at advanced stages of AML. Flow cytometry showed a 2-fold reduction of total cells and a 4-fold increase in HSPCs in AML-chip compared to BMME-chip. In addition, marked reduction of osteocalcin in AML-chip at day 7 and day 14 via gene expression analysis (41.10 ± 13.50% and 16.60 ± 5.00% respectively compared to BMME-chip) indicate a loss of osteoblastic activity similar to *in vivo* results. This finding is supported by the apparent decrease in mineralization of the osteoblastic cell layer in AML-chip at day 7 observed via alkaline phosphatase and von-kossa staining. Strikingly, we found a significant increase of CCL3 level in the effluent of AML-chip at 918.49 ± 192.54 pg/mL compared to BMME-chip at 498.73 ± 79.05 pg/mL via ELISA assay. Our results so far indicate that the AML-chip can recapitulate the disease phenotypes reported *in vivo* and supports the reliability of our model to generate a human AML-chip.

Presentation: Oral

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Building blocks for cognition-in-a-dish: Brain MPS, electrophysiology and expression of molecular machinery of learning and memory

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The human brain is unmatched in its energy-efficiency and ability to perform tasks currently out of reach for Artificial Intelligence. To combine the best of both worlds, an interface between modern information technology and biological material must be created. With the advances in high-density multi electrode arrays (MEAs), a suitable biological model needs to be developed to elucidate interaction on MEAs.

Recent advances in brain cell cultures bring this goal closer to realization. Our group has developed a 3D brain microphysiological system (BMPS) derived from induced pluripotent stem cells which consists of differentiated mature neurons, astrocytes, and oligodendrocytes (Pamies et al., 2017). The BMPS reproduces neuronal-glial interactions and connectivity and shows synaptogenesis and neuron-to-neuron interaction, e.g. as spontaneous electric field potentials. These critical elements of neuronal function show its potential as a unique brain-machine interface towards biological computing, but further research is needed to understand the molecular processes and electrophysiology of the BMPS in development and learning.

Immediate Early Genes (IEGs) have been shown to be crucial for synaptic plasticity (Okuno, 2016) and thus learning. Therefore, we characterized our BMPS during different stages of its development by quantifying the expression of those IEGs (*ARC*, *EGR1*, *FOS*, *BDNF*, *NPAS4*, *NPTX2*), together with other genes involved in synaptic plasticity and learning (transcription factor *CREB1* and *SYNGAP1*). We also included microRNAs, involved in finetuning of synaptic plasticity (*miR-124-3p*, *miR-132-3p* and *miR-134-5p*). miRNAs as well as *ARC*, *NPAS4*, *NPTX2*, *FOS* and *CREB1* were induced during BMPS differentiation, while *SYNGAP1* and *EGR1* were expressed more consistently throughout the differentiation. *BDNF* was upregulated at early stages of differentiation and went down in more mature BMPS. We stimulated the BMPS with agonists and antagonists of GABA and GLUTA receptors and assessed the response of the IEGs, demonstrating an increase of *ARC*, *NPAS4* and *FOS*. Linking to functional response, electrophysiological parameters, e.g., evoked and spontaneous electrical field potentials of the BMPS, were recorded using Calcium imaging and high-density MEAs. The latter was

additionally used to induce electrical stimulations and detect resulting changes in electrophysiology.

Currently, we are using Immunohistochemistry to visualise and verify localisation of IEG proteins.

Presentation: Poster

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ASTEROIDS: Coupling organoid culture with a multicellular MPS using a dynamic and novel platform

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Emergence of organoids and organ-on-chip technologies have recently provided two advanced cancer models by their capacity, (1) to recapitulate the three-dimensional (3D) architecture and heterogeneity of the tumor, and (2) to reproduce its multi-cellular biochemical and mechanical environment, respectively. Although complementary, these technologies have not been combined yet into a more physiologically relevant human model. Therefore, we recently developed a new microphysiological system (MPS) as an Apparatus to Simulate Tumor and Reproduce Organs in an Interactive and Dynamic System (ASTEROIDS). This plastic device is comprised of an optically transparent polycarbonate center piece consisting of six wells for the culture of 3D organoids. The center piece is separated by two porous membranes on each side and connected to two side chambers for the culture and perfusion of vascular and stroma compartments. The ASTEROIDS ergonomics and design provide opportunity for organoid live imaging, simple disassembly for membrane and organoid collection, throughput with 96-well plate format compatibility, and extreme modularity allowing a multi-device configuration (i.e., "body-on-chip"), thus providing a robust and versatile platform adapted for a large range of bioassays performed by any biomedical scientists without engineering background. The data showed the ASTEROIDS platform can sustain the co-culture up to 7 days and the cells even demonstrated establishment and maintenance of key tissue features such as endothelial barrier function or tumor pathophysiological gradients. In addition, perfusion of immune cells showed they could attach to activated endothelial cells in the vascular chamber under physiological flood velocity and cross the barrier to reach the lung spheroid. Finally, the ASTEROIDS has been subjected to X-Ray radiation and results demonstrated increase of DNA damage and repair, cell junction disruption and immunological cell death initiation, suggesting that cells within the ASTEROIDS are able to respond to external stimuli. On-going studies are currently assessing the potential of ASTEROIDS platform to reproduce key hallmarks of *in vivo* tissue and provide a novel step towards more relevant *in-vitro* human tissue system for drug screening and testing or personalized medicine.

Presentation: Poster

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3D neural interfaces on chip

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Tissue engineered microphysiological systems (MPS) have recently emerged as a more patient-relevant and cost-effective alternative for disease modeling and drug discovery compared to traditional cell culture and animal models. Despite their increased functional relevance, most current MPS lack capabilities for analysis of individual components or layers through biological assays. Furthermore, current MPS rarely use 3D cell cultures to accurately mimic biological structures.

Here, we present a preliminary nerve-organ MPS. This novel MPS includes three removable layers: a three-chamber glass-bottom 3D cell culture layer which allows the contact of different cell types with GelPins (previously published by our lab), with the middle 3D chamber in contact with a 2D cell culture layer through a semi-permeable membrane, and a removable media reservoir at the top of the assembly. The MPS also includes separate pipetting access ports to each individual chamber, which allows the specific addition and removal of cell culture medium, treatments, or reagents. This design allows specific endpoint analyses for each chamber, including immunostaining for the 3D layer, and western blotting, flow cytometry, PCR, and immunostaining for the 2D layer. Furthermore, the glass bottom of the 3D hydrogel layer allows for high-resolution light and fluorescent microscopy of both the 2D and 3D layers, while the layers are assembled or separated.

The manufacturing and assembly of the MPS includes the use of a stereolithography (SLA) 3D printing, laser cutting, and layer-by-layer assembly (as previously published by our lab). To form the connecting, three-chamber cell culture layer, an SLA-printed clear resin piece is attached to a glass coverslip to form the bottom of the assembly. We have demonstrated this method of assembly to include cardiac and adrenal systems inclusive of the autonomic nervous system (ANS) to better mimic human biology. Further, sympathetic neurons induce a clear change in cardiac behavior compared to control. We hypothesize that organ function is reliant upon the ANS and paramount to the design of an accurate MPS. Our presentation will outline our approaches for successfully including the ANS.

Presentation: Poster

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Cell shape dominates over physiological substrate stiffness by limiting nuclear localisation of the fibrogenesis gatekeeper, YAP/TAZ

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Micropatterning allows for fine-tuned microenvironmental control over factors such as spatial constraints and orientation in both three-dimensional and two-dimensional platforms. The feasibility of controlling both cell orientation, shape and substrate stiffness becomes crucial in modelling diseases that are highly governed by tissue stiffness, such as Idiopathic pulmonary fibrosis (IPF). IPF is a disease known to be accompanied by the augmentation of matrix stiffness caused by aberrant deposition and the remodelling of extracellular matrix. The stiffening of the lungs enhances Transforming Growth Factor-β (TGF-β) positive feedback loops by activating latent TGF-β, resulting in irreversible pulmonary damage [1]. Polyacrylamide gels with strictly controlled shapes printed on substrate of tunable stiffness enables multidimensional disease modelling in cell culture. Current work explores the relationship between cell shape, substrate stiffness and TGF-β treatment with the use of novel micropatterning methods on polyacrylamide gels. Collagen micropatterns of 500 μm^2 were created by using UV-C range light (189 nm) on glass and polyacrylamide substrates. MRC-5 human embryonic lung fibroblast cell line was seeded on the substrate to create single cell arrays. Immunofluorescence staining revealed the effects of microenvironmental factors, such as cell aspect ratios (AR) and substrate stiffness on the location and abundance of Yes-associated protein/transcriptional coactivator with a PDZ-binding domain (YAP/TAZ). Under stiff glass condition, substrate stiffness dominates the effects of cell shape, showing distinct YAP/TAZ translocation to nucleus. In contrast to the extremes of substrate stiffness, TGF-β appeared to induce YAP/TAZ nuclear localization at 5 kPa only in elongated (AR = 5) cells. Conversely, rounded cells (AR = 1) displayed an attenuation of TGF-β YAP/TAZ nuclear localisation, suggesting a potential effect of cell shape on TGF-β and YAP/TAZ pathway. Our results show that a tipping point between the effects of cell shape and substrate stiffness exists, such that mechanotransduction mechanism has the potential to slow the progression of IPF by shifting fibroblastic-like cells towards anti-fibrogenic phenotype.

Reference

[1] Barratt, S. L., Creamer, A., Hayton, C. et al. (2018). Idiopathic pulmonary fibrosis (IPF): An overview. *J Clin Med 7*, 201.

Presentation: Poster

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Microphysiological systems for studying enteric neuron-epithelial interactions

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Introduction: Microphysiological systems (MPS) provide a physiologically relevant model with broad applications including the Gut-Brain-Axis. In addition, the relationship between the nervous system and epithelium lining the gut is understudied in inflammatory gut disorders and neurogenerative dysfunction such as Parkinson's Disease (PD). PD is defined as alpha-synuclein (a-Syn) aggregates spreading centrally, and the gut-origin hypothesis of PD is gaining traction. Therefore, a model system to study PD transmission in an MPS would benefit discovery.

Materials and methods: MPSs were assembled with the laser cut and assembly with polycarbonate, semi-permeable PET membranes, and 3M acrylic adhesive (Hosic et al.; Soucy et al.). A contacting enteric neuron (EN) culture from rat myenteric plexus was encapsulated below an epithelial cell (EC) monolayer derived from rat small intestinal organoids. EC were seeded on a collagen-coated membrane layer at day 4, while EN were seeded on day 0. Transepithelial resistance (TEER) and Lucifer yellow (LY) assays were performed over 10 days for EC membrane permeability. In 2D, fluorescent a-Syn preformed fibrils (PFFs) were applied to EN to mimic accumulation in PD for 7 days, dosed with 1 μg of PFF, and cultured for 14 more days with 2D electrophysiology with multielectrode arrays (MEA). Endpoint imaging followed.

Results and discussion: EC+EN MPSs showed tight junction formation (ZO-1) and chromogranin (ChgA) immunolabeling suggesting enterocyte and enteroendocrine cell differentiation from the primary EC. TEER values fluctuated with a decrease on day six. By day 10 the TEER values and LY were similar and aligned with the physiological range in mice, indicating a stable culture. We also found EN uptake PFF to the neural soma over 21 days and aggregation as a model of gut-PD in a dish and found that dosing short-chain fatty acid Butyrate rescued accumulation. No functional changes were apparent with electrophysiology (not shown; Bindas, 2023). These studies show the applicability of MPS for interrogation of the ENS on gut health, and more extended studies of PFF and microbiota impact in other disease geometries are underway.

We thank Northeastern University, HDDC, and the NIH and NSF funding.

Presentation: Poster

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Using weighted gene correlation networks analysis to explore similarities and differences in genetic regulatory networks in glioma, iPSC brain organoids, and CNS-derived immortalized cell lines

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Understanding the molecular mechanisms of neurodegenerative diseases has been challenging for many reasons, including the lack of correspondence between *in vitro* models and *in vivo* processes and the difficulty of obtaining sufficient patient-derived tissue samples with adequate power for a true systems biology approach.

Here, we use multiple high-powered, transcriptomic data sets from CNS-derived tissue: gliomas sequenced as part of the TCGA (The Cancer Genome Atlas), iPSC derived models of neurons and astrocytes, and a compendium of transcriptomics data from CNS-derived cell lines from the ARCHS4 gene expression database to explore some of the key differences between the *in vivo* and *in vitro* data sets.

WGCNA (Weighted Gene Correlation Network Analysis) – which uses graph theory to derive genetic regulatory networks from high-dimensional transcriptomic data – is a sensitive way to delineate key differences between regulatory networks; our prior work has shown that using this approach demonstrated significant differences between patient-derived breast cancer tissue samples from TCGA and MCF-7 cells, an immortalized breast cancer cell-line, as well as the limitations of using a cell-line derived from a single individual that fails to capture the genetic diversity of human populations. Using this approach on multiple data sets of CNS-derived tissue, we identified both similarities and differences in several pathways relevant for neurodegenerative diseases and cancer, including circadian rhythm regulation, myelin sheath formation, and inflammation.

Presentation: Poster

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Pancreatic islet (PANIS) microphysiological system for modeling type 2 diabetes

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Diabetes is an increasingly prominent disease affecting 537 million people worldwide with type 2 diabetes (T2D) comprising over 90% of cases. T2D progression correlates with toxic bioenvironment due to glucolipotoxicity resulting accelerated β-cell dysfunction and impaired insulin secretion. With the development of a T2D MPS model, high content testing can be implemented in therapeutics that can aid in expediting the transition from initial testing to clinical trials.

In this study, we developed an islet-MPS using both primary human islets and human induced Pluripotent Stem cell (hiPSC) derived organoids to mimic the T2D disease state. The Pancreatic Islet (PANIS) system is a glass-based 3-layer 2-chamber design with the middle glass layer housing a porous polyester membrane encased in a commercially available organ-on-chip platform with dedicated fluidic ports for vascular and parenchymal chamber perfusion. Human islets encapsulated in hydrogel are micropatterned on this membrane prior to assembly and perfusion to maintain islet 3D morphology and mimic interstitial flow.

Primary islets retained high viability (> 95%) and glucose stimulated insulin secretion (GSIS) over 28 days of culture (stimulation index (SI) from 3 mM to 16 mM glucose > 2). Islet phenotype was maintained with ≈70% C-peptide+ and ≈21% Glucagon+ cells. The progression of T2D was modeled by simulating diseased states of glucotoxicity, lipotoxicity, and glucolipotoxicity with long term exposure to pathological glucose (20 mM) and free fatty acid concentrations (0.5 mM oleic and palmitic acid). The resulting islets showed lower C-peptide cells (≈60%) and loss in GSIS (SI≈1). Secretome from disease conditions show elevated CCL2 (3-6-fold) and IL-8 (> 20-fold) which are involved in inflammation and immune cell recruitment; increase in VEGF secretion (2-4 fold) corresponding with CD31+ cells (25% of islet volume) and ROS expression (4-fold increase). Transcriptomic analysis in simulated disease conditions is being compared to reported T2D patient islet-transcriptome to evaluate the biomimet-

ic disease model. Parallel efforts with hiPSC derived organoids showed GSIS responsiveness (SI > 1.7) over a 14-day period. Single cell RNA-sequencing of iPSC-islets on PANIS demonstrate activation of key pathways over static culture. Current efforts are underway to mimic T2D condition with iPSC-islets.

Presentation: Poster

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Fully automated high-throughput drug toxicity evaluation on the hematopoietic niche in a bone marrow model

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Over the last decade, New Alternative Methods (NAMs) such as human organs-on-chips have been developed to overcome the clinical translation shortcomings and ethical issues associated with animal experimentation. However, current approaches are harshly limited by low throughput, lack of reproducibility and intensive manual labor. These issues limit their use to specific niches within preclinical efficacy and safety screening cascades and prevented their widespread adoption and implementation by the pharmaceutical industry. To address these issues, we developed a high-throughput platform infrastructure with end-to-end robotic automation for the seeding, cultivation, dosing, imaging, and multi-omic analysis of thousands of independent, functional, self-organizing, vascularized 3D human tissue models in parallel. As an example, we present an integrative bioengineering strategy to develop a vascularized, microphysiological human bone marrow model that leverages the ability of adult stem cells to self-organize into a complex, specialized microenvironment of human hematopoietic stem/progenitor cells (HSPCs). The microengineered niche reconstitutes key characteristics of native human bone marrow such as HSC self-renewal at the vascular niche, multilineage hematopoiesis, and complex ligand-receptor signaling pathways, with each individual tissue containing over 300,000 cells and independently perfusable through a self-assembled 3D microvascular network. The generation of functionally mature myeloid cells allows interrogation of key physiological processes of innate immunity such as neutrophil chemotaxis and intravascular mobilization. Receptor-ligand interaction analysis with scRNA-seq confirmed that intercellular signaling critical to the maintenance of the HSPC niche was recapitulated, including KIT-KITLG, CXCR4-CXCL12, and NOTCHx-DLLx interactions. Treatment with clinically relevant concentrations of FDA-approved anticancer drugs demonstrated concentration-dependent induction of erythropenia, neutropenia and thrombocytopenia predictive of clinical human results, as quantified in our models by phenomic, proteomic, and transcriptomic analysis of thousands of independent bone marrow tissues. Subsequent treatment with G-CSF resulted in recovery of the hematopoietic niche following treatment. In addition to bone marrow, the fully automated platform has the capability to cultivate multiple different human tissue models for the evaluation of pharmaceutical agents. In summary, the platform supports the high-throughput interrogation of complex *in vitro* models that faithfully recapitulate complex human phenotype and function, while also providing robotic reproducibility and walk-away automation to a formerly artisanal, effort-intensive process.

Presentation: Oral

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Cellular co-culture rather than 3D environment improves cardiomyocyte functionality in gellan gum-gelatin hydrogel

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In traditional 2D *in-vitro* models, the characteristic mechanisms and architecture of cardiovascular systems are difficult to recapitulate. Encapsulating cells in three-dimensional (3D) extracellular matrix (ECM) can provide mechanical stimulus and topography required by cardiomyocytes (CM), reflecting the physiological tissue microenvironment more than stiff cell cultures. The integration of patient-specific induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) allows easy generation of tissue-like preparations and modelling of patient-specific genetic mutations. We have previously shown that vascular structures support the maturation of human induced Pluripotent Stem Cell derived Cardiomyocytes (iPSC-CM) in 2D culture [1] and in this study we developed two different multicellular cardiac constructs in gellan gum-gelatin hydrogel presenting structural and mechanical support for the beating CMs in 3D environment.

In myocardial coculture we combined human adipose stem/stromal cells (hASCs) with iPSC-CMs and in the cardiovascular multiculture, human umbilical vein endothelial cells with fluorescent tag (GFP-HUVEC, Cellworks) and hASCs were combined with iPSC-CMs. Within these multicellular constructs, cells were seeded inside and on top of hydrazone crosslinked gelatin-gellan gum hydrogel [2]. 2D and 3D iPSC-CMs were used as controls.

We compared the functionality of iPSC-CMs to detect the most optimal environment for key cardiac functions with each construct using video microscopy and calcium imaging assays. Our results demonstrate that both constructs notably improved CMs morphology and contractility compared to 2D and 3D iPSC-CM controls.

References

[1] Vuorenpää, H., Penttinen, K., Heinonen, T. et al. (2017). Maturation of human pluripotent stem cell derived cardiomyocytes is improved in cardiovascular construct. *Cytotechnology 69*, 785-800. doi:10.1007/s10616-017-0088-1

[2] Gering, C., Párraga, J., Vuorenpää, H. et al. (2022). Bioactivated gellan gum hydrogels affect cellular rearrangement and cell response in vascular co-culture and subcutaneous implant models. *Biomater Adv 143*, 213185. doi:10.1016/j.bioadv.2022.213185

Presentation: Poster

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3D bioprinted vascularized tumor model for triple-negative breast cancer disease modeling

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More than 90% breast cancer related deaths are due to cancer cell metastasis to secondary organs. Triple negative breast cancer is highly aggressive and lacks targeted therapeutics. The tumor microenvironment complexity is difficult to recapitulate using conventional techniques such as two-dimensional (2D) cell culture and three-dimensional (3D) *in vitro* models comprising spheroid cultures, scaffold-based models, and organ on a chip, which are inefficient in recreating the complexity of the tumor microenvironment concerning cellular spatial localization, density, vasculature, and 3D complexity. 3D bioprinting can address this issue by incorporating several cells along with vasculature mimetic channels in a high throughput manner to recreate the tumor microenvironment with more *in vivo* relevance and reproducibility.

Stereolithography-based 3D bioprinting has gained significant attention due to the ability to recreate complex structures such as tubular geometries with high fidelity, mechanical stability, and moderate printing conditions. Vascularized breast cancer tumors can provide better alternatives for immunotherapeutics screening and disease modeling. To recreate the vascularized triple-negative breast cancer tumor model (highly invasive cancer) by using visible light-based digital light processing (DLP) based bioprinter, in this research work, we have developed bioinks with different polymers such as Poly(ethylene glycol) diacrylate 700 Da (PEGDA), gelatin methacrylate (GelMA), PEG dimethacrylate 8kDa (PEGDM) and optimized the printing parameters for various wt.% combinations GelMA with PEGDA or PEGDM based bioink for vascular channel bioprinting. A combination of GelMA and PEGDM based bioink printing was optimized with high cell viability and proliferation for MDA-MB-231 cells for 7 days. The compressive modulus for printed structures was 73kPa, which is closer to invasive ductal carcinoma tissue. The rheological characterization indicated high linear viscoelastic regions and long-term stability for printed constructs. The physiological flow conditions impact on the cell-laden microfluidic channel will be elucidated to understand the impact of shear stress on the cancer cells. In future studies, the 3D model complexity will be increased by endothelialization and immune cell infiltration for better *in vivo* relevance.

Presentation: Poster

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Applicability assessment of human immortalized cell-based blood-brain barrier models for characterization of brain permeability of cyclic peptides

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Treatment of CNS disorders requires drugs that can pass through the blood-brain barrier (BBB), and, therefore, BBB permeability evaluation of candidate drugs using human BBB models plays an important role in identifying promising ones. Accordingly, we have developed a spheroidal BBB model based on human immortalized cells, but it remains to be evaluated how useful the models are in BBB permeability studies of drugs. Therefore, we here sought to clarify applicability of the spheroidal BBB models to drug BBB permeability studies through characterization of the kinetic properties of the SLS peptide, a BBB-permeable cyclic peptide [1].

The spheroidal BBB models were developed using human immortalized astrocytes (HASTR/ci35), pericytes (HBPC/ci37), and brain microvascular endothelial cells (HBMEC/ci18) as reported previously [2]. Using the FAM-labeled SLS peptides (10 μM), we first analyzed the temperature-dependency (37°C vs 4°C) of its uptake into the BBB spheroids at 60 min by semi-quantitatively

measuring the fluorescence intensity inside the spheroids. The results showed the uptake levels were 7.0-fold higher at 37°C than those at 4°C, suggesting involvement of a putative carrier-mediated pathway in its BBB permeability. When analyzed in the ranges of 10-360 min, this carrier-mediated uptake levels showed a saturation trend, which was initiated at around 60 min. We also found that, while the carrier-mediated uptake levels at 40 min increased in a concentration-dependent manner, no saturation could be seen in the range of 3-30 µM.

To summarize, we have characterized the uptake properties of the SLS peptides using the spheroid BBB models and found that a relatively low-affinity carrier-mediated mechanism is involved in its BBB penetration. Therefore, it can be expected that the spheroidal BBB models are useful for evaluation of the BBB permeability profile of cyclic peptides, presumably as well as other macromolecule drugs.

References

[1] Yamaguchi et al. (2020). *J Control Release 321*, 744-755.
[2] Kitamura et al. (2022). *Mol Pharm 19*, 2754-2764.

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Physical coupling of a vascularized human liver acinus microphysiological system (vLAMPS) and pancreatic islet microphysiological system (PANIS) to recapitulate progressive hepatic insulin resistance and the systematic causal link to type 2 diabetes (T2D)

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Nonalcoholic fatty liver disease (NAFLD) is a global health problem with a comorbidity of 70% with Type 2 diabetes. In the presence of excess circulatory free fatty acids (FFAs), the endocrine pancreas and liver axis play a major role in the cascade of systemic insulin resistance, hyperglycemia, and hyperinsulinemia. The onset of hepatosteatosis leads to the failure of pancreatic-islet β-cells causing insulin scarcity, completing the cycle of T2D [1]. Attempts to identify the mechanistic bridge between targetable hepatokines and β-cell failure is challenging due to the systemic nature of *in-vivo* models.

The complex interplay between organ-specific crosstalk can be examined through coupled Liver-Islet MPS systems. Our MPS platform enables us to recapitulate and study the NAFLD-T2D pathophysiology by mimicking tissue-specific microenvironments and facilitating high-content analysis in a two-module platform. Our upstream module is comprised of a vascular Liver Acinus Microphysiological System (vLAMPS) that recapitulates a portocentral zonation with a space of Disse and fenestrated vascularization [2]. Downstream the Pancreatic Islet microphysiological system (PANIS) is comprised of a parenchymal space capable of prolonged sustainability of primary human pancreatic islets. The integrated platform allows compartment-specific readouts from the common flowing medium.

To determine the basal viability of our connected system we implemented a chemically defined normal fasting media (NFM) [3]. 10-day perfusion in NFM showed retention of primary islet function of Glucose Stimulated Insulin Secretion (GSIS) and preservation of healthy α and β-cell distribution. The liver parenchyma remained sensitive to glucose and insulin with low signs of steatosis. The increase of glucose and introduction of FFAs to the media leads to the recapitulation of early metabolic syndrome (EMS). Upstream we observed increased steatosis and fibrosis, with decreased insulin sensitivity. Downstream, we recorded decreased GSIS and disrupted β-cell populations. Our multicompartment MPS allows us to screen for an array of macromolecules from specific spatiotemporal events between organs, specifically, to facilitate the identification of NAFLD derived hepatokines that lead to β-cell dysfunction and T2D.

References

[1] Tilg, H. et al. (2017). *Nat Rev Gastroenterol Hepatol*.
[2] Gough, A. et al. (2021). *Nat Rev Gastroenterol Hepatol*.
[3] Saydmohammed, M. et al. (2021). *Exp Biol Med*.

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Modeling the interaction between tumor spheroids and vasculature using on-chip vascular bed platform

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Background: Three-dimensional (3D) tissues offer a physiologically-relevant model for pharmaceutical screening. However, their lack of functional vasculature restricts their size and developmental maturity. This issue has been addressed through the integration of 3D tissues with on-chip vasculature constructed via self-organizing processes, such as vasculogenesis and angiogenesis. In most models, 3D tissues are required to provide angiogenic factors for the induction of vascular formation and are embedded in a hydrogel, which limits the application of 3D tissues to on-chip vascular models. To overcome this limitation, we propose a novel technique for culturing the 3D tissue directly on pre-formed vasculature termed as "on-chip vascular bed (VB)." VB is constructed using growth factors or co-culturing with human lung fibroblasts (hLFs). The tissue is settled on the hydrogel, which can be adapted to any culture environment, such as an air-liquid interface condition. Thus, the VB system expand the adaptability of 3D tissues. In this study, we used two kinds of spheroids for the demonstration: hLF spheroids and alveolar soft part sarcoma (ASPS) spheroids.

Method: A five-channel device was utilized with the tissue culture well placed above the center channel [1]. Initially, the tissue culture well and the center channels were separated by a membrane. After VB was constructed in the center channel using HUVECs, the membrane was removed to expose the VB to the well. Subsequently, the spheroid was introduced on the VB.

Results: Four days after the co-culture of VB and the hLF spheroid, VB was connected to the spheroid. This connection was also observed in the case of the ASPS spheroid. Moreover, the introduction of a fluorescent dye into VB revealed its flow into the ASPS spheroid, indicating that the VB system enables the administration of drugs to the spheroids via VB. ASPS cells migrated to the VB region and attached to the VB wall, demonstrating the direct contact between the spheroid and VB promoted their interaction.

Reference

[1] Kameda, Y. et al. (2022). *Lab Chip*.

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Human cortical neuronal networks in microelectrode embedded microphysiological system to study functional alterations during alpha-synuclein aggregation and propagation as model for Parkinson's disease

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Alpha-synuclein (a-syn) is a protein mainly located in synapses taking role in synaptic transmission [1]. Aggregated forms of a-syn is a pathological hallmark of Parkinson's Disease (PD). During the aggregation process, a-syn gains a toxic function and/or loses its normal function leading to reduction of synaptic proteins, progressive impairments in neuronal excitability, synaptic activity, network connectivity, and eventually, neuronal death [2]. Studying α-syn pathology and its effects on functionality is critical for understanding the mechanisms behind disease development and progression. However, conventional *in vitro* models are not suitable to track the propagation of aggregated forms of a-syn, whereas microfluidics technologies provide more accurate models for both cellular and functional level dissection [3].

Here, we studied the functional perspective of aggregation and propagation of a-syn in human induced pluripotent stem cell-derived engineered neuronal networks. Physically separated human neuronal networks connected to each other only via axons extended through microtunnels; thus, enabled to study proximal and distal effects during the spread of pathology. Also, embedded microelectrode arrays (MEAs) allowed studying functional markers during the process. The spread of pathology between networks was successfully tracked with immunocytochemistry. In parallel to MEA recordings, mitochondrial motility in the axons and calcium activity was assessed together with pre-synaptic protein quantity and cell viability in different phases of aggregation.

Results indicated some functional or structural changes were seen in different phases of aggregation: decrease in mitochondrial functioning or reduction of presynaptic proteins. Alterations in neuronal activity was not a clear indicator in the model, however, networks containing pathological a-syn formations responded divergently to glutamate receptor modulators which was only detectable in engineered networks.

In conclusion, the proposed aggregation/propagation model for PD provides temporal data on functional vs structural changes during the spread of pathology. Further improvement of the mod-

el would provide functional screen for validation of the candidate drugs and open the way for precision medicines in PD studies.

References

[1] Bendor, J. T. et al. (2013). The function of α-synuclein. *Neuron 79*, 1044-1066.
[2] Soto, C. (2003). *Nat Rev Neurosci 4*, 49-60.
[3] Pelkonen, A. et al. (2020). *Biosens Bioelectron 168*, 112553.

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A high-throughput, 28-day, microfluidic model of human gingival tissue inflammation and recovery

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Gum disease, comprising mild (gingivitis) to severe (periodontitis) inflammation of the gingival tissue, effects nearly half of American adults aged 30 years or older [1]. Although one of the world's most prevalent diseases, oral health research remains limited by the tools and technology available to evaluate oral pathophysiology and its impact on systemic health and disease. The absence of available and practical research tools has hindered advances in understanding oral inflammation and the ability to screen oral therapeutic and prophylactic agents *in vitro*. Here, we present a high-throughput, 28-day, microfluidic gum-on-chip model within the Draper PREDICT96 platform (known as MOUTH) composed of human gingival tissue grown in a dynamic microenvironment as a promising approach to address these shortcomings. Consisting of primary oral keratinocytes, gingival fibroblasts and endothelial cells, the tri-culture model displays physiological structural and functional features including stratified tissue layers, mucosal barrier formation, and protein biomarker secretion over several weeks. We demonstrate the induction of inflammation within MOUTH through inflammatory cytokine administration as measured by changes in barrier function and cytokine secretion. Inflammation can be induced at various time points within a stable culture window and resolved via delivery of small molecule inhibitors, providing a robust platform for evaluation of therapeutic agents. These data reveal that the administration of specific small molecule inhibitors mitigates the inflammatory response and enables tissue recovery, permitting an opportunity to identify new therapeutic targets for gum disease with the potential to facilitate relevant preclinical drug efficacy and toxicity testing. This high-throughput and biomimetic microphysiological tissue culture platform, MOUTH, provides complex and responsive human tissue at scale, thereby reducing a potential reliance on animal models for oral health and disease research.

Reference

[1] Division of Oral Health, National Center for Chronic Disease Prevention and Health Promotion, Adult Oral Health (2020). https://www.cdc.gov/oralhealth/basics/adult-oral-health/index.html

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Liver-on-a-chip: Development of patient-specific liver models utilizing iPSCs and novel microfluidic chip devices

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Hepatocytes carry out many essential functions of the human liver including synthesis of cholesterol and detoxification of numerous substances. To overcome challenges associated with primary human hepatocytes and cancer cell lines in liver *in vitro* modeling, induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) can be used. Here, we differentiate iPSC-HLCs by a slightly modified three-step protocol originally published by Kajiwara et al. [1] The conventional 2D *in vitro* liver models are useful to some degree, but we aim to proceed to the next level with our patient-specific iPSC-HLC 3D on-chip cultures, which will have enhanced functionality and longevity compared to 2D liver models.

Here we used novel microfluidic chip devices developed at the University of Gothenburg (PI Caroline Adiels), that allow 3D culture and media perfusion while minimizing the shear stress on the cells. They have previously reported a liver-lobule-on-a-chip with promising results [2]. The current devices are designed specifically to mimic liver-specific microenvironment and consist of 21 individual liver lobules connected through media flow. After an initial 5-day culture in 2D, the cells are mixed with a hydrogel and seeded on the chips to continue their differentiation in 3D culture conditions under continuous media flow.

When comparing the expression of liver-specific markers on d15 and d20 on-chip, 3D cultures had a higher expression of mature hepatocyte markers ALB and A1AT at the later time point.

Concurrently, the expression of CK19, a marker of liver progenitor cells, decreased over time in the 3D cultures. On the contrary, in 2D control cultures, the expression of both A1AT and ALB decreased and the expression of CK19 increased over the same period, suggesting their dedifferentiation. Combined with data from ELISA assays, our results suggest the novel device supports 3D culturing of differentiating iPSC-HLCs. In the future, we aim to establish similar models using iPSC lines produced from coronary artery disease patients with the goal to explore the production of lipids and lipoproteins and the effects of inflammatory factors on these in different patient groups.

References

[1] Kajiwara, M. et al. (2012). *Proc Natl Acad Sci 109*, 12538-12542.
[2] Banaeiyan, A. A. et al. (2017). *Biofabrication 9*, 015014.

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Lung-mimicking hydrogel culture systems to study host-pathogen interaction and drug efficacy in tuberculosis

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Mycobacterium tuberculosis (Mtb) is an obligate human pathogen and can survive in host macrophages. The bacteria infect resident macrophages upon reaching the alveolus of hosts, and the subsequent inflammation cascade leads to the formation of a three-dimensional (3D) granuloma [1].

Two-dimensional (2D) *in vitro* mammalian cell infection with Mtb is simplified and not representative of the cell environment in the body. Mice are the principal animal model being used but do not get infected by Mtb, the same way as humans. Notably, lung pathology (granuloma formation) and disease phenotypes differ. Other animal models (such as guinea pigs) are either not representative of human disease or require specialized facilities (for non-human primates). To overcome these challenges, we have been working to develop three-dimensional (3D) *in vitro* models.

We have engineered collagen I hydrogels to infect THP-1 monocytes, or peripheral blood-derived mononuclear cells (PBMCs), with Mtb. Infecting THP-1 monocytes with Mtb in collagen gels resulted in a significantly longer window to study host-pathogen dynamics than conventional macrophage infection studies carried out in 2D. The THP-1 cells in the infected collagen gels recapitulated characteristics of human TB infection phenotypically and genotypically. We also found pyrazinamide, a first-line anti-tuberculosis drug, to be effective in reducing the bacterial load (by 2 log orders at the clinically relevant concentration range), not shown in any *in vitro* model thus far [2].

TB granulomas are complex pathologies that pose a challenging environment (hypoxia, necrosis), which Mtb can overcome. Hence to further understand the host-pathogen dynamics in this environment, we infect human PBMCs with Mtb in collagen gels in custom-designed bioreactors to mimic the human lung interstitial dynamics. The bioreactor has been designed to facilitate the formation of physiologically relevant 3D hypoxic necrotic TB granulomas, and we have reached cellular aggregates of 200 mm. These models have the potential to be next-generation platforms for accelerating TB drug discovery and development.

References

[1] Ramakrishnan, L. (2012). Revisiting the role of the granuloma in tuberculosis. *Nat Rev Immunol 12*, 352-366. doi:10.1038/nri3211
[2] Gupta, V. K. et al. (2023). Lung-mimicking hydrogel culture system reproduces several tuberculosis phenotypes and demonstrates pyrazinamide efficacy. *bioRxiv*, 2023.2001.2024.525291. doi:10.1101/2023.01.24.525291

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A predictive multi-organ-chip platform for cancer precision medicine using automated high-content substance testing

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Since cancer is a condition that greatly varies between individuals, there is no universal approach of treatment. Patient-derived tumor models are already being developed to enable personalized drug treatments. However, patient-specific modelling of drug metabolization needs more complex test systems that can combine different organ models. Microphysiological systems (MPS) offer a possible solution for this obstacle, as they are able to emulate the interconnected human physiology. To allow for the necessary standardization and throughput required for patient testing, systems are needed that enable highly standardized automated handling of MPS. Here we use the HUMIMIC AutoLab to test drug treatment on human cancers. This system is a powerful automatization tool that allows

handling of 24 multi-organ-chips 24/7 including continuous fluorescent and brightfield imaging. To demonstrate the methodology, two different cancer types were investigated: Patient derived gastric tumor organoids and diffuse large B cell lymphoma cell lines. To test the individual responses to chemotherapeutic prodrugs, a co-culture of each tumor model with a 3D liver spheroid model composed of HepaRG and human hepatic stellate cells for drug metabolism was established in a multi-organ-chip. Morphologic, metabolic and immunohistochemical analysis showed that the co-cultures were stable in the multi-organ-chip for at least 7 days. For the gastric cancers, the data showed effectiveness of two 5-Flourouarcil prodrugs, Tegafur and Capecitabine, commonly used in the therapy of gastric cancer, indicating biotransformation of the prodrug to their toxic metabolites via the liver model. This resulted in a significantly decrease in the gastric cancer biomass of two individual patients. For the diffuse large B-cell lymphoma cell lines, data showed varying sensitivities of the cells when treated with biotransformed Cyclophosphamide, dependent on the original cancer subtype and genetic background of the patient. The individual results of chemotherapy treatments create a path for combination and novel therapies, resulting in prognoses that are more precise and subsequently increases individual patients' treatment success.

Presentation: Poster

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PDMS-free gut on a chip as a tool for patient-derived anaerobic microbiota research

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The gut microbiota has a critical role in human health and are involved in many if not all physiological and pathological processes, including cancer development. However, there is still a limited knowledge how exactly gut microbiota can contribute to cancer development. Bacterial derived extracellular vesicles (BEV) play a significant role in it since they can transfer microbiota derived signal molecules. Currently, research of human gut microbiota communication mechanisms with host cells is complicated and research methods for these processes are limited. Therefore, the aim of our research is to study cancer patient microbiota derived BEV content, which can enter from gut lumen to circulation by applying vascularised GoC model. This approach could be used as potential biomarker identification tool for dysbiosis in future. To that end, we have currently developed a GoC device suitable for anaerobic microbiota cultivation and successfully optimised anaerobic microbiota isolation and cultivation in GoC from human stool samples. Next, we cultivated cancer patient microbiota within GoC functionalised with stable and primary cell lines for > 72 h and performed metagenome analysis, BEV analysis in gut lumen and endothelial channel, followed by dynamic epithelial/endothelial barrier integrity analysis. Results showed that majority of bacteria are still strictly anaerobic or anaerobic after cultivation with our chip, however several most common bacterial strains were changed depending on patient after cultivation suggesting for selective pressure from model. Finally, BEV from gut lumen and those that can pass through gut-endothelial barrier were collected, analysed by NTA and BEV RNA content was analysed. Data showed that different composition of microorganisms in GoC based on metagenome data affects biological barrier permeability and BEV transport from gut to endothelial channel in comparison to control. While BEV RNA analysis revealed which microorganism derived BEV are predominantly secreted and transported during GoC cultivation.

Presentation: Oral

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Human-induced pluripotent stem cell reporters for high-content screening of stress response activation identifying target organ-specific toxicities

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The development and validation of next generation *in vitro* test systems to improve the prediction of chemical-induced adversity for specific target organs is critical. Human-induced pluripotent stem cells (hiPSCs) are a valuable source for studying chemical-induced toxicities in various organ specific cell types while having the same genetic background. This enables to identify organ specific toxicities during chemical safety screening. The activation of stress response pathways is one of the early key events leading to organ injury. Monitoring critical genes within these pathways would both give insight in the mode-of-action as well as aid in the prediction for liabilities for adverse outcomes upon exposure. Here, using the CRISPR/Cas9 technology we have built a reporter panel in hiPSCs in which carefully selected genes based on transcriptomic analysis for critical stress response pathways were endogenously tagged with eGFP. The hiPSC reporter panel covers inflammatory signaling (VCAM1, IRF1, A20 and LCN2), DNA damage (p21),

ER stress (CHOP, TRIB3, CHAC1, HYOU)1, and oxidative stress (PIR, SRXN1, HMOX1) response pathways. These reporters were validated by characterizing reporter functionality, genomic stability, and their pluripotency. The activation of stress response pathways was monitored using live cell confocal imaging upon chemical exposure in multiple cell types after differentiation (e.g. hepatocyte-like cells, proximal tubular-like cells, cardiomyocytes). Differences in sensitivity towards chemical exposure between different organ specific cell types could be observed. To allow to study multi-cellular dependent responses and the utility of more advanced 3D systems, these reporters can be grown as organoids for both the liver and kidney. In kidney hiPSC-derived reporter organoids, DNA damage activation was seen upon cisplatin exposure specifically in proximal tubular cells but not in glomerular cells. This allows to study cell specific responses within a multi-cellular environment more recapitulating the *in vivo* settings. We anticipate that these stress response hiPSC reporters will aid in improving chemical safety testing allowing for the identification of the mode-of-action as well as specific target organ or cell specific toxicities.

This work has received funding from the EC Horizon2020 EU-ToxRisk project (grant number 681002) and the EC Horizon2020 RISK-HUNT3R project (grant number 964537).

Presentation: Oral

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Uncovering SARS-CoV-2 pathogenic insights and screening therapeutics in a reproducible and high-throughput BSL3 human airway-on-chip platform

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COVID-19 emerged as a worldwide pandemic early in 2020 and since then has caused over 753 million cases and over 6.8 million deaths worldwide [1]. Over the past 3 years, several SARS-CoV-2 variants and subvariants have emerged, causing varying degrees of infectious waves across the globe. Rapid evaluation of emerging variants remains critical for understanding pathogenic implications, working towards new medical countermeasures, and informing public response. Efficacious therapeutic compounds remain severely needed, yet the process for identifying treatments has been slow and arduous. Promising candidates identified by immortalized cell lines often fail to demonstrate efficacy when progressed to animal models and humans. This dilemma highlights the need for new drug screening technologies that can parse drug candidates early in development with regard to predicted relevance for clinical use.

We present PREDICT96-ALI, a high-throughput microfluidic organ-on-chip platform composed of human primary bronchial epithelial cells grown at an air-liquid interface in a dynamic tissue microenvironment. We demonstrate successful viral replication of live SARS-CoV-2 in multiple donors and with multiple variants (Washington, Delta, Omicron). PREDICT96-ALI is able to resolve differential efficacy in five antiviral compounds over a range of drug doses and predict clinical outcomes of SARS-CoV-2 drug treatments (nirmatrelvir, molnupiravir, and remdesivir), demonstrating the value of the technology as a prognostic drug discovery tool. Complementary viral genome quantification and immunofluorescence microscopy readouts achieve high repeatability between devices and replicate plates, highlighting the technology's reproducibility. These data suggest that PREDICT96-ALI can be used as an antiviral screening tool for SARS-CoV-2, combining the high-throughput functionality of a 96-well plate format in a high containment, Biosafety Level 3 (BSL-3) laboratory with the relevant biology of primary human tissue.

Reference

[1] World Health Organization, WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/ (2023, January 31).

Presentation: Oral

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Novel fabrication strategies to reduce the presence of inert materials inside microphysiological systems

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All the tissues in our body are extremely well organized. The cell-matrix and the cell-cell interactions are crucial for the different cell types to preserve their physiological phenotype. This aspect has been a significant drawback during the last decades because no *in vitro* models could replicate the proper cellular distribution. The appearance of the microphysiological systems has allowed, with their channel and chamber designs, to resemble the histological distribution *in vitro* faithfully. Despite the vast improvement achieved with this technology, some limitations remain. One of the main ones is the presence inside the microfluidic chips of inert materials (PDMS or plastic membranes), separating

two different channels/chambers. Those inert membranes avoid direct contact between cells and matrix but also introduce a rigid structure in the model affecting the physiological cell behaviour. The main goal of this work has been to reduce as much as possible the presence of inert materials inside the microfluidic chip, preserving the proper cellular distribution.

To achieve this goal, we have designed two microfluidic devices with an inert membrane with macropores that allow near total (> 75%) contact between its sides.

The first chip was designed to resemble the cell-matrix interaction with almost no interference from the inert materials. This design contains an inert membrane of 4 mm in diameter, with the capacity for three macropores of 1 mm each. This pore size was optimized to its maximum, considering the viscosity of the hydrogels (collagen I) used.

A second chip was also designed, but in this case, to mimic the cell-cell interaction without any hydrogels. We fabricated a chip containing a nylon mesh with regular pores of 150 microns to achieve this. In this case, the protocol requires the preculture of the cells as spheroids with a diameter of around 250 microns. Ones the spheroids were formed, they were seeded over the nylon mesh allowing the cells to migrate and covert all the pores of the device. After the monolayer formation, a second cell type can be seeded to create the cell-cell direct interaction.

Presentation: Poster

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The vascularized micro-tumor (VMT): A fully human microphysiological system platform for testing multiple immuno-oncology therapies

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Cancer immunotherapies have demonstrated remarkable results but have been efficacious in only a small (10-20%) subset of patients with solid tumors. Unfortunately, testing new strategies has been slowed by the species-specific nature of many of the reagents and cells that are being developed, which often do not behave in mice as they do in humans. To provide an alternative to mice we have optimized the fully human Vascularized Micro-Tumor (VMT) platform [1-3] and shown its effectiveness for testing multiple immunotherapy modalities. The VMT consists of a microvascular network connecting a microfluidic "artery" and "vein" through which flows a blood substitute. Tumors are established around this network allowing for nutrients and drugs to be delivered to the growing tumor in a physiologic manner. CAR-T cells introduced to the input wells flow through the vessels where they can adhere to the vasculature and extravasate in an ICAM-1-dependent process that mirrors *in vivo* leukocyte trafficking. We find that killing of targets under these conditions is extremely specific, with non-detectable killing by control cells, in sharp comparison to 2D assays where there is often considerable bystander killing by non-targeted cells. The vasculature in the VMT is leakier than that seen in control tissues without tumors, which show physiologic permeability, allowing for rapid delivery of ADCs to the tumor cells, where specific tumor killing is again observed. Finally, recruitment of the endogenous immune system, in the presence or absence of bi-specific antibodies can also be assessed in the VMT platform by perfusion with PBMC that are either autologous, MHC-matched or fully allogeneic. In summary, the VMT provides an exciting new platform that is fully human cell-based and that provides a useful adjunct to, and in some cases replacement for, studies in mice.

References

[1] Moya, M. L. et al. (2013). In vitro perfused human capillaries. *Tissue Eng 19*, 730-737.

[2] Sobrino, A. et al. (2016). 3D microtumors in vitro supported by perfused vascular networks. *Sci Rep 6*, 31589.

[3] Hachey, S. J. et al. (2021). An in vitro vascularized micro-tumor model of human colorectal cancer recapitulates in vivo responses to standard-of-care therapy. *Lab Chip 21*, 1333-1351.

Presentation: Poster

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Assessment of risk factors in chronic kidney disease using proximal-tubule microphysiological systems

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Chronic kidney disease (CKD) is a global killer affecting over 850 million people worldwide. In the last 30 years, low/middle income countries have seen a drastic rise in CKD despite patients not having diabetes or hypertension. A major health burden these regions, this chronic kidney disease of unknown etiology (CKDu) is now thought to be a result of multiple environmental agents. The pathophysiological processes that lead to CKDu progression are poorly understood, and in this work proximal tubule microphysiological systems (PT-MPS) are employed to examine the role of ochratoxin-A (OTA), an environmental mycotoxin, in nephrotoxicity and CKDu.

Chronic exposure of OTA is a concern as OTA is thought to accumulate in the proximal tubule, though its mechanism(s) are not well understood. RNA-seq analysis of human primary proximal tubule epithelial cells (PTECs) treated with OTA in 2D culture revealed that OTA dysregulates several key genes involved in a number of biochemical pathways including the NRF2-dependent antioxidant response, and interestingly the hypoxic response through the induction of transcription factor HIF1a and numerous genes regulating mitochondrial dynamics. This was corroborated in PT-MPS as tubules treated with OTA under a simulated low oxygen environment through coadministration cobalt chloride revealed substantial HIF1a nuclear stabilization and cytotoxicity seen with immunocytochemistry staining. These results highlight their susceptibility to excess hypoxic stress. Confocal microscopy of PTECs in 2D culture treated with OTA under low oxygen conditions further corroborated mitochondrial dysfunction, where excessive fission, mitophagy and swollen mitochondria were observed under treatment conditions. To evaluate this phenomenon further in relation to tubular nephrotoxicity, PT-MPS will be treated with OTA, cobalt chloride in the presence and absence of STING inhibitors. RNA sequencing, injury biomarkers such as KIM-1 and NGAL, and levels of pro-inflammatory cytokines will be evaluated to assess the role of this pathway in OTA-nephrotoxicity. Finally, vascular-proximal tubule MPS will be used to model OTA renal toxicity by analyzing its basolateral uptake, tubular accumulation, and apical efflux in the presence and absence of relevant renal transporter inhibitors. This data will be used to develop a physiologically-based pharmacokinetic (PBPK) model of OTA renal clearance in humans.

Presentation:: Oral

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Bioinspired microfluidic chip for vascularized multi-niche bone marrow

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Current studies on bone marrow mostly rely on the use of two-dimensional and three-dimensional (3D) cell cultures and animal models, that do not necessarily reflect the exact physical, chemical, and biological characteristics of bone marrow niche. Under these circumstances, recent studies are emerging with Organ-on-chip microfluidic devices that deliver a favorable alternative for the research of cell behavior in a more accurate environment [1,2]. However, the complexity of bone marrow microenvironment limits the opportunity to recapitulate the physiological and pathological conditions effectively, thus breaking open prospects for an ideal bone marrow model.

In this regard, our objective was to explore the utility of high throughput microfluidic technology towards creating a novel bone marrow on chip model involving major cellular and extra cellular constituents of bone marrow niche. We could develop an optically accessible microfluidic chip using 3D-printing method that enabled rapid construction of 3D microstructures. The printed device consists of multiple channels designed to accommodate various niche components of the bone marrow, perforated inner walls that allow a hydrostatic pressure derived interstitial flow among the channels without the use of external pumps and shafts connecting different channels which are predesigned to form physical compartmental barriers, while exchanging media, nutrients, and cellular secretions. The cell cultures studies with optimal ratio of various cells and media demonstrated that the experimental setup could mimic the physiological microenvironment. The presence of the interconnected vascular networks (which is one of the most essential characteristics of the functional bone marrow) in the vascular compartment further extends the prospective of our developed device. Thus, we believe that our current bone marrow chip model has the potential to replicate natural marrow structure and function in a more realistic manner when compared to the previous studies. Further, once optimized, this chip in combination with patient derived cells can emerge as an ideal model for personalized research and drug screening.

References

[1] Glaser, D. E et al. (2022). Organ-on-a-chip model of vascularized human bone marrow niches. *Biomaterials 280*.

[2] Yu, J. et al. (2022). Perfusable micro-vascularized 3D tissue array for high-throughput vascular phenotypic screening. *Nano Converg 9*, 16.

Presentation: Poster

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Structural and functional impact of co-culturing iPSC-CMs and HCAEC within microfluidic devices exposed to mechanical and electrical stimuli

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According to the World Health Organization cardiovascular diseases take the lives of 17.9 million people every year, 31% of global deaths. Cardiomyocytes (CMs) derived from induced pluripotent stem cells (iPSCs) have a strong potential in regenerative medicine, disease modelling and pharmacological studies. However, iPSC-CMs generated through current methods are immature, in structure and function, presenting characteristics of fetal CMs. Im-

mature CMs exhibit disorganized sarcomere structures, weakened contraction, spontaneous beating, improper calcium handling and electrophysiological signalling, low expression of critical cardiac proteins and altered responses to drugs when compared with mature CMs. Several methods have been developed to induce maturation of iPSC-CMs, including dimensionality, substrate stiffness, biochemical exposure, long term culture, co-culture with other cell types, mechanical stress and so on [1].

In this work, we propose the co-culture of iPSC-CMs with human aortic endothelial cells (HCAECs) on a perfusable COP-based microfluidic device with two culturing channels communicated through a porous membrane. Pure monolayers of immature CMs and endothelial cells were grown on those channels while electromechanical stimulation was applied for 10 days. Then, in order to assess the culturing scheme, we studied the cardiac contractility with an image-based method and the sarcomere formation as well as the electrical response, the calcium transient and the junction formation.

Reference

[1] James, E. C., Tomaskovic-Crook, E. and Crook, J. M. (2021). Bioengineering clinically relevant cardiomyocytes and cardiac tissues from pluripotent stem cells. *Int J Mol Sci 22*, 3005.

Presentation: Poster

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Monitoring drug-induced nephrotoxicity modified by cell polarity in renal proximal tubule epithelial tissue with an impedance measurement system

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Drug-induced nephrotoxicity is one of the most common adverse events reported during the drug development (Wilmer et al., 2016). While it accounts for 2% of the failures in the drug development in the non-clinical tests, it reaches 19% of the failures in the clinical tests (Naughton, 2008). The main failure in drug development is the reliance on the non-clinical tests on animal models because human physiology is fundamentally different from the animals (Saio et al., 2000).

To overcome this issue, kidney MPS have been widely utilized to physically and chemically mimic the human *in-vivo* environment (Kimura, 2018). The MPS model of the proximal tubule, which is a segment of the nephron, is of particular interest for nephrotoxicity studies because the proximal tubule epithelial tissue has a variety of membrane transporters that are asymmetrically located at the apical and basal sides and mediate active clearance, reabsorption, and local interstitial accumulation of drugs (Perazella, 2009).

Although the proximal tubules constantly transport drugs and the dynamics of drug nephrotoxicity need to be assessed, the assessment is typically based on end-point viability assays such as live/dead and MTT assays which require labels and limit prognostic information (Xing et al., 2005). In addition, these assays can only assess advanced stages of kidney injury elicited by high doses of the drug (Soo et al., 2018). Few proximal tubule MPS models currently incorporate real-time monitoring to assess drug-induced nephrotoxicity.

Electrical measurement techniques can assess complex physiological states in real time because they are label-free and non-invasive methods (Xu et al., 2016). Here we show that impedance measurements can be applied for the real-time detection of the drug-induced subtle nephrotoxicity.

We first designed a bi-channel microfluidic device integrated with electrodes. It was demonstrated that measurements of impedance can help in monitoring the differences of cisplatin-induced nephrotoxicity depending on the polarity of membrane transporters in the RPTECs and have the potential to predict nephrotoxicity. The proposed MPS is a useful tool for the nephrotoxicity assessment of novel candidate compounds for drug development.

Presentation: Poster

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Bioprinted hydrogel-based microphysiological systems recapitulating the cellular crosstalk in tissue barriers

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Tissue barriers have a crucial role in regulating homeostasis. They are the first line of defense against external insults and the exchange site of nutrients and other molecules. Endothelial and epithelial barriers ensure tissue compartmentalization, supported by the stromal compartments that contain a variety of cell types. Moreover, most of these tissue barriers have defined 3D structures such as the crypt-villous structures of the small intestine or the tubular shape of the blood vessels that modulate cell behavior. All these features are essential for cellular crosstalk between different compartments that regulates tissue functionality and has major implications in diseases such as infection or cancer. However, tissue barriers models accounting for the complex 3D architecture, the stromal compartment, and the soft mechanical properties of the tissue, while maintaining the compatibility with routine cell culture techniques are scarce due to the limitation of standard biofabrication tools.

Here we present a fast, low cost and versatile approach for the fabrication of complex tissue barrier models by means of digital light projection stereolithography (DLP-SLA). By employing optimized bioink formulations of low macromer content, based on gelatin methacryloil (GelMA) and poly-(ethylene glycol) (PEGDA) hydrogels, and an accurate selection of the printing parameters (energy dosages and exposure times), we produced functional 3D structures with soft mechanical properties, containing both the barrier and the stromal compartments. The printed tissues, compatible with standard cell culture and conventional *in vitro* testing techniques, were kept in co-culture for long culture time periods. Embedded stromal cells such as fibroblasts (human intestinal fibroblasts or mouse 3T3) or immune cells (THP-1 monocytes or Jurkat T-cells) showed excellent cell viability (> 95%). The tissue surrogates sustained the growth of epithelial and endothelial cells, that formed an effective barrier. Our models evidenced a cellular crosstalk between the different compartments. Epithelial cells for example influenced the distribution of embedded fibroblasts, resembling the one found *in vivo*, as well as the differentiation state of encapsulated monocytes. Thus, our 3D bioprinting approach enables the fabrication of functional tissue barrier surrogates that recapitulate not only the 3D architecture but also the native stromal microenvironment of the *in vivo* tissue.

Presentation: Poster

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An immune-competent microvascularized human lung-on-a-chip device for studying immunopathologies of the lung

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Advances in microphysiological organ-on-chip technologies have enabled spatiotemporal investigation into the biology of organ systems in healthy and disease-like conditions *in vitro*. The highly-tunable nature of on-chip models permit direct manipulation of the microenvironment and provides the framework to study disease progression in a way not feasible through other *in vitro* or *in vivo* models. Here, we describe the development of a two-layer human lung-on-chip (LOC) device to study the lung immune landscape, a continuation of work described in [1]. The LOC consists of an interstitial niche with perfusable microvasculature, lung fibroblasts, and resident immune cells, and an epithelial layer at air-liquid interface separated by a membrane. The devices are fabricated in a 96-well format to increase throughput and allow for integration with liquid handling and automated imaging tools. To recapitulate the immune landscape, the interstitial layer contains tissue-resident interstitial macrophages and dendritic cells, the epithelial layer contains airway-like macrophages, and circulating immune cells in the form of PBMCs or RBC-depleted whole blood are perfused through the vascular network. We found that interstitial macrophages localized perivascularly, while macrophages introduced into the airway layer adopted a more airway macrophage-like phenotype, with a significant increase in CD11c and HLA-DR. Currently, the immune-competent lung-on-chip device is being used to evaluate the lung response to H1N1 infection. Through the modular use of the LOC, we investigated the role of specific immune populations in the infection response. We observed that upon infection, the immune LOC exhibited an increase in IL-6, IL-8, and IL-12p70 as well as IFN-λ and IFN-β. However, the magnitude and kinetics of the responses varied significantly depending upon the incorporated immune populations. Beyond cytokine release profiles, fluorescence microscopy was used to evaluate the effect of H1N1 infection on the spatial organization of the LOC. It was found that tissue-resident macrophages reduced the overall disease severity within the LOC by up-taking viral particles, demonstrated by a mitigation of H1N1-induced endothelial and epithelial damage. Overall, we have developed an immune-competent human LOC device that is capable of recapitulating the lung-immune landscape in both healthy and disease states.

Reference

[1] Mejias (2020). *Lab Chip 20*, 3601-3611.

Presentation: Oral

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Utilizing long-term potentiation in iPSC-cortical neurons to investigate CBD and echinacea as treatments for chronic stress

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The induction of stress results in an increase of the stress hormone cortisol in the brain. While acute cortisol exposure is hypothesized to cause hyperexcitation or synaptic enhancement in the CNS, chronic levels of cortisol drive neuronal fatigue and synaptic decline downstream from the preceding hyperexcitation of the neural network [1]. This phenomenon appears clinically through chron-

ic fatigue and cognitive dysfunction. Long-term potentiation (LTP) serves as an *in vitro* correlate for memory and learning and can be measured through enhanced neuronal activity akin to synaptic strength. Human-derived iPSC-cortical neuronal networks cultured on microelectrode arrays (MEAs) were utilized to investigate acute and chronic stress at low and high levels. Chronic low levels of cortisone (0.1 μM) showed synaptic enhancement, while chronic high levels of cortisone (10 μM) resulted in synaptic deterioration preceded with synaptic suppression at the medium level (1 μM). After establishment of this CNS microphysiological platform for stress in the CNS, the efficiency of Echinacea, along with one of its Dodeca ingredients, were evaluated for their ability to mitigate stress-induced LTP deficits. Following a chronic, 7-day treatment with 1 μM hydrocortisone, neural networks were evaluated for synapse integrity. LTP maintenance was evaluated at 4, 24, and 48 hours following the induction of LTP via high-frequency stimulation. CBD was used as positive controls for stress mitigation. Dodeca showed a dose-dependent rescue of LTP maintenance compared to the vehicle control condition. Furthermore, Echinacea displayed rescue capabilities similar to that of the positive control CBD. This study suggests that Echinacea can be used as a potential therapeutic to counteract chronic-stress induced cognitive impairment.

Reference

[1] Popoli, M., Yan, Z., McEwen, B. S. et al. (2011). The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci 13*, 22-37. doi:10.1038/nrn3138

Presentation: Poster

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Lung-mimicking hydrogel culture systems to study host-pathogen interaction and drug efficacy in tuberculosis

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Mycobacterium tuberculosis (Mtb) is an obligate human pathogen and can survive in host macrophages. The bacteria infect resident macrophages upon reaching the alveolus of hosts, and the subsequent inflammation cascade leads to the formation of a three-dimensional (3D) granuloma [1].

Two-dimensional (2D) *in vitro* mammalian cell infection with Mtb is simplified and not representative of the cell environment in the body. Mice are the principal animal model being used but do not get infected by Mtb, the same way as humans. Notably, lung pathology (granuloma formation) and disease phenotypes differ. Other animal models (such as guinea pigs) are either not representative of human disease or require specialized facilities (for non-human primates). To overcome these challenges, we have been working to develop three-dimensional (3D) *in vitro* models.

We have engineered collagen I hydrogels to infect THP-1 monocytes, or peripheral blood-derived mononuclear cells (PBMCs), with Mtb. Infecting THP-1 monocytes with Mtb in collagen gels resulted in a significantly longer window to study host-pathogen dynamics than conventional macrophage infection studies carried out in 2D. The THP-1 cells in the infected collagen gels recapitulated characteristics of human TB infection phenotypically and genotypically. We also found pyrazinamide, a first-line anti-tuberculosis drug, to be effective in reducing the bacterial load (by 2 log orders at the clinically relevant concentration range of drug), not shown in any *in vitro* model thus far [2].

TB granulomas are complex pathologies that pose a challenging environment (hypoxia, necrosis), which Mtb can overcome. Hence to further understand the host-pathogen dynamics in this environment, we infect human PBMCs with Mtb in collagen gels in custom-designed bioreactors to mimic the human lung interstitial dynamics. The bioreactor has been designed to facilitate the formation of physiologically relevant 3D hypoxic necrotic TB granulomas, and we have reached cellular aggregates of 200 mm. These models have the potential to be next-generation platforms for accelerating drug discovery and development.

References

[1] Ramakrishnan, L. (2012). Revisiting the role of the granuloma in tuberculosis. *Nat Rev Immunol 12*, 352-366. doi:10.1038/nri3211

[2] Gupta, V. K. et al. (2023). Lung-mimicking hydrogel culture system reproduces several tuberculosis phenotypes and demonstrates pyrazinamide efficacy. *bioRxiv*, 2023.2001.2024.525291. doi:10.1101/2023.01.24.525291

Presentation: Poster

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ECG findings and clinical presentations of myocardial ischemia reported among patients with cardiac metastasis from lung malignancies: A narrative review

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Cardiac tumours are substantially infrequent. However, metastasis to the heart from a primary cancer elsewhere in the body is reported often. In addition to its poor prognosis, the diagnosis of a cardiac metastasis is considered tough to establish. Primary lung cancers contribute to the maximum of cardiac metastasis cases. Owing

to its predominantly clinically silent nature, myocardial metastasis isn't usually detected until autopsy. This narrative review aims at highlighting the ECG findings that are seen among patients with myocardial metastasis resulting from lung cancer. It also analyses the clinical presentations associated with cardiac metastasis. Although ECG findings are not standard means of diagnosis, characteristic changes were reported, which might suggest further investigations for the same. The studies reported in this review were collected from the databases that include PubMed, ScienceDirect, Hindawi, ResearchGate and AHA journals in the period of 1980-2022. The keywords used for searching in the databases included ECG, cardiac metastasis, lung cancer. Articles focusing on lung cancer specifically was included, and studies reporting findings associated with other forms of cancer were excluded. A majority of case reports was used for this review. Literature review showed that ECG findings in a patient with cardiac metastasis imitated that of myocardial infarction. This review article encourages health researchers to decipher and justify the findings reported and develop a quicker strategic outline for diagnosis. It also aims to educate the healthcare professionals on the early detection of myocardial metastasis with the study of the preliminary ECG picture, thereby ensuring a better prognosis.

Presentation: Poster

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Bioengineering of human vascular networks with controlled geometry using DLP bioprinting

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Tissue engineering holds great promise for regenerative medicine, drug discovery and as an alternative to animal models. However, as soon as the dimensions of engineered tissue exceed the diffusion limit of oxygen and nutriments, a necrotic core forms leading to irreversible damage. To overcome this constraint, the establishment of a functional perfusion network is essential and is a major challenge to be met [1].

In this work, we explore a promising Digital Light Bioprinting approach [2] to encapsulate endothelial progenitor cells (EPCs) in 3D light-cured hydrogel scaffolds to guide them towards vascular network formation. We observed that EPCs encapsulated in the appropriate photopolymerized hydrogel can proliferate and self-organize within a few days into branched tubular structures with predefined geometry, forming capillary-like vascular tubes or trees of various diameters (in the range of 10 to 100 µm). Presenting a monolayer wall of endothelial cells strongly connected by tight junctions around a central lumen, these structures are stable for several weeks *in vitro*. Interestingly, our technology has proven to be versatile in promoting the formation of vascular structures using a variety of vascular cell lines, including EPCs, human vascular endothelial cells (HUVEC) and human dermal lymphatic endothelial cells (HDLEC).

We have also demonstrated that these vascular structures can be recovered and manipulated in an alginate patch without altering their shape or viability. This opens new opportunities for future applications, such as stacking these endothelial vascular structures with other cell sheets or multicellular constructs to yield bioengineered tissue with higher complexity and functionality.

References

[1] Wang, Z., Mithieux, S. M. and Weiss, A. S. (2019). Fabrication techniques for vascular and vascularized tissue engineering. *Adv Healthc Mater 8*, e1900742.

[2] Mazari-Arrighi, E., Ayollo, D., Farhat, W. et al. (2021). *Biomaterials 279*, 121207.

Presentation: Poster

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Digital light bioprinting of an *in vitro* self-renewing corneal limbal epithelial model

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The cornea serves as a protective barrier for the eye and facilitates light refraction onto the retina. Regeneration of the cornea's epithelium ultimately derives from the limbal epithelial stem cells (LESCs) found in the limbus, a transition zone surrounding the cornea. Damage to this area can eventually lead to vision loss. Considering the significant shortage of cornea donors, as well as lack of disease models allowing the discovery of novel therapeutic approaches, effective alternative reconstructive therapies are needed [1].

To this end, the project presented here aims to microfabricate the unique topography of alternating ridges and crypts that recapitulate the native environment of LESCs, namely the Palisades of Vogt, to restore the structure and function of the corneal epithelium *in vitro*. Photocrosslinkable hydrogels consisting of collagen methacrylate (ColMA) and methacrylated hyaluronic acid (HAMA), which are naturally found in the native cornea, serve as cell scaffolds.

Using Digital Light Processing technology, UV light is projected in a grayscale manner onto the hydrogel, with varying doses producing different crosslinking densities and stiffness gradients.

Notably, seeded corneal epithelial cells show selective adher-

ence, spreading, and better monolayer formation on hydrogel areas exposed to higher UV doses. Moreover, timelapse microscopy suggests that these cells migrate along a UV gradient – from low to high-exposed areas, revealing a potential mechanotactic behavior. Furthermore, the hydrogel supports the culture of corneal epithelial cells up to at least 3 weeks, with simultaneous partial differentiation and stratification as shown by immunofluorescence confocal microscopy.

By exploiting UV dose control, we envision the generation of distinct limbal and corneal environments that promote stemness, or migration/differentiation respectively [2], creating a self-renewing model suitable for long-term *in vitro* studies. Furthermore, a microfluidic system is currently being developed with perfusion of medium and air to generate "air-lifting", for complete primary epithelial cell maturation and stratification [3]. The same chip will serve as a drug screening platform to evaluate the pharmacological relevance of this limbal-corneal model.

References

[1] Mobaraki et al. (2019). *Front Bioeng Biotechnol 7*, 135.
[2] Gouveia et al. (2019). *Nat Commun 10*, 1496.
[3] Yu et al. (2022). *iScience 25*, 104200.

Presentation: Poster

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Scalable application of RosetteArray™ technology for modeling the complex etiology of human neural tube defects and screening for risk factors

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Neural tube defects (NTDs) remain the second most common congenital malformation. Given their complex omnigenic and environmental etiology, rodent models have limited utility for investigating NTD pathophysiology and screening for prophylactic interventions. Alternatively, human pluripotent stem cell (hPSC)-derived neural rosettes model *in vivo* neurulation and can be used in precision medicine screens. Using foundational hPSC neural differentiation [1,2] and bioengineering [3] protocols, we developed RosetteArray technology to standardize neural rosette derivation in a micropatterned 96-well plate screening format and combine it with adaptive confocal imagining and AI-based image analysis to create a transformative platform for developmental neurotoxicity (DNT), modeling NTD etiology, and screening for novel NTD prophylactics. Here, we present the platform's capability to detect pharmaceuticals, agrochemicals, and genetic mutations with known clinical NTD risk. First, reproducible derivation of forebrain and spinal cord RosetteArrays from direct seeding of cryopreserved cells is demonstrated. Second, we present a preliminary DNT screen (24 pesticides and 6 NTD-associated substances) in which the RosetteArray performs with 91% sensitivity and 100% specificity and includes integration of simulated human metabolism. Third, we describe differential responses between forebrain and spinal RosetteArrays to a teratogen and a genetic NTD risk factor, thereby supporting inclusion of both region-specific assays in future screens for more comprehensive coverage of neural tube morphogenesis. Fourth, we present modeling of a clinically-relevant multifactorial NTD scenario where a mutant hPSC line with a NTD genetic predisposition only shows a risk phenotype in the presence of an environmental risk factor. Lastly, we discuss ongoing experiments using iPSC lines derived from patients with Spina Bifida, the most prevalent clinical NTD, to develop a precision medicine prophylactic screen. Collectively, these results support scalable implementation of the RosetteArray platform for investigating NTD etiology, conducting DNT screens to identify risk factors, and developing precision medicine approaches for discovering novel NTD risk-reducing prophylactics.

COI: RSA, GTK, and RMW are co-founders of Neurosetta LLC, which is commercializing RosetteArray technology.

References

[1] Lippmann, E. S. et al. (2015). *Stem Cell Reports 4*, 632-644.
[2] Iyer, N. R. et al. (2022). *Sci Adv 8*.
[3] Knight, G. T. et al. (2018). *eLife 7*, e37549.

Presentation: Oral

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Development of a microphysiological skin-liver-thyroid Chip3 and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions

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All cosmetic ingredients registered in Europe must be evaluated for their safety using non-animal methods. Microphysiological systems (MPS) offer a more complex higher tier model to evaluate chemicals. Having established a skin and liver HUMIMIC Chip2 model demonstrating how dosing scenarios impact the kinetics of chemicals, we investigated whether thyroid follicles could be incorporated to evaluate the potential of topically applied chemicals to cause endocrine disruption. This combination of models in the HUMIMIC Chip3 is new; therefore, we describe here how it was optimized using two chemicals known to inhibit thyroid production, daidzein and genistein. The MPS was comprised of Phenion® Full Thickness skin, liver spheroids and thyroid follicles co-cultured in the TissUse HUMIMIC Chip3. Endocrine disruption effects were determined according to changes in thyroid hormones, thyroxine (T4) and 3,3',5-triiodothyronine (T3). A main part of the Chip3 model optimization was the replacement of freshly isolated thyroid follicles with thyrocyte-derived follicles. These were used in static incubations to demonstrate the inhibition of T4 and T3 production by genistein and daidzein over 4 days. Daidzein exhibited a lower inhibitory activity than genistein and both inhibitory activities were decreased after a 24 h preincubation with liver spheroids, indicating metabolism was via detoxification pathways. The skin-liver-thyroid Chip3 model was used to determine a consumer-relevant exposure to daidzein present in a body lotion based on thyroid effects. A "safe dose" of 0.235 µg/cm^2, i.e., 0.047% applied in 0.5 mg/cm^2 of body lotion was the highest concentration of daidzein which does not result in changes in T3 and T4 levels. This concentration correlated well with the value considered safe by regulators. In conclusion, the Chip3 model enabled the incorporation of the relevant exposure route (dermal), metabolism in the skin and liver, and the bioactivity endpoint (assessment of hormonal balance, i.e., thyroid effects) into a single model. These conditions are closer to those *in vivo* than 2D cell/tissue assays lacking metabolic function. Importantly, it also allowed the assessment of repeated doses of chemical and a direct comparison of systemic and tissue concentrations with toxicodynamic effects over time, which is more realistic and relevant for safety assessment.

Presentation: Oral

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Mantarray 3D engineered muscle tissue platform demonstrates clinically-relevant disease stratification of an *in vitro* Duchenne muscular dystrophy model

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Introduction: Accurately modeling healthy and disease conditions *in vitro* is vital for the development of new treatment strategies and therapeutics. For cardiac and skeletal muscle diseases, direct assessment of contractile output is a reliable metric to study overall tissue function, as other "proxy" measurements are poor predictors of muscle strength. Human 3D engineered muscle tissues (EMTs) from induced pluripotent stem cell and primary cell sources hold great potential for modeling contractile function. However, the bioengineering strategies required to generate these predictive models presents limitations for many investigators.

Methods: Here, we have developed a platform that utilizes 3D EMTs in conjunction with a label-free magnetic sensing array (Mantarray). The platform enables facile and reproducible fabrication of 3D EMTs using virtually any cell source and is coupled with individual, well-based control of stimulation and highly parallel direct measurement of contractility. This approach enables clinically relevant functional measurements of muscle, stratification of healthy and diseased muscle phenotypes, and facilitates compound safety and efficacy screening.

Results: We present a 3D model of Duchenne muscular dystrophy that utilizes skeletal muscle EMTs formed from an isogenic pair of healthy and diseased cells. These tissues achieve robust twitch and tetanic responses upon stimulation. The model presents functional deficits across numerous metrics of contractility, including force and fatigability. EMTs remain functional for weeks to months in culture and provide a large experimental window to not only study therapeutic effect, but also disease phenotypes that may present at later stages of development and maturity. We have also established a method to suspend these EMTs in a biocompatible gel that permits transfer of tissues between labs. Tissues remain viable and fully functional upon gel dissolution for direct interrogation with therapeutic compounds, eliminating the need to fabricate EMTs in-house.

Conclusions: These data demonstrate a first-and-only commercial platform integrating individual, well-based control of electrical stimulation across a 24-well plate to pace 3D tissues, modeling exercise regimens or damage protocols in muscle constructs. Stimulation is coupled with automated assessment of 3D muscle contraction, providing an inclusive, high-throughput platform for disease modeling and therapeutic discovery.

Presentation: Oral

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Highly biomimetic 3D bioprinted tubular small intestine model leads to *in vivo*-like differentiation of human adult stem cell-derived organoids

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In recent years, *in vitro* models have gained popularity as a fast and effective way to better understand disease pathogenesis and to further drive progress in therapeutics [1]. Specifically, intestinal models with high *in vivo*-fidelity could provide important support in tackling some of the most common disorders and shed light on key pathways linking together different parts of our body in healthy and diseased states. However, current solutions fail to aptly recapitulate the different traits found in the human gut, be that due to the absence of the various intestinal cell types, lack of geometrical, cellular, or mechanical cues or missing peristalsis-like flow and air-liquid interface conditions [2].

Herein, we developed a human-derived 3D small intestine tissue model through microfluidic bioprinting of single-layered hollow tubes using decellularized porcine small intestinal submucosa and sodium alginate that is functionally and structurally comparable to its native counterpart. This coaxial bioprinting technology exploits the instantaneous ionic crosslinking between sodium alginate and calcium chloride resulting in strong and stable hollow tubes with an average inner diameter of 500 µm and average wall thicknesses of 60 µm. Our 3D bioprinting approach allows us to recapitulate not only the protein composition of the substrate found *in vivo* but also the tubular geometry of the human intestine. Moreover, this extrusion system allows us to obtain a continuous high-throughput fabrication of perfusable tubes that can be adjusted in length and cross-sectional area. We leveraged these highly biomimetic structures to induce the formation of an epithelial tube-shaped monolayer from adult stem cell-derived intestinal organoids, thus proving the key role that scaffold geometry plays in stem-cell differentiation. The potential of our approach opens the door for more complex tissue constructs that can include additional physiological and diseased stimuli such as flow-induced shear stress, cell-cell interactions, and multi-organ-on-chip systems.

References

[1] Adhikary, P. P., Ul Ain, Q., Hocke, A. C. et al. (2021). COVID-19 highlights the model dilemma in biomedical research. *Nat Rev Mater*. doi:10.1038/s41578-021-00305-z

[2] Pimenta, J. et al. (2022). Organ-on-chip approaches for intestinal 3D in vitro modeling. *Cell Mol Gastroenterol Hepatol 13*, 351-367.

Presentation: Poster

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How adipoids can help us in immune metabolism research

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Adipose tissue is associated with some of the most common causes of death worldwide: cardiovascular diseases, cancer and diabetes. Immune cell-adipocyte crosstalk is known to drive these but so far, we are lacking a good model system to study these interactions. To study adipose tissue-related diseases *in vitro*, classical 2D cell culture systems are coming to their limitations. 2D cultured adipocyte-like cells, like 3T3 or differentiated primary fibroblasts, show a flat architecture and often multilocular lipid droplets, which don´t resemble the round monocular adipocytes found in white adipose tissue. Furthermore, they lack the extracellular matrix- necessary for cell-cell communication- and contact with other cell types, like the immune cells. Suitable 3D adipose tissue spheroid models

often lack resident immune cell populations and therefore require the addition of immune cells and specific growth factors. We have created the first 3D adipose tissue organoid of the stromal vascular fraction that can autonomously regulate the development of resident immune cell populations without adding external growth factors, which is more reflective of the *in vivo* situation. We demonstrated by lipidomic analysis that this model reflects the physiological lipidome better than traditional 2D cultures, making it a more convenient tool for studying immune metabolism as well as dietary and immunomodulatory interventions. We recently recapitulated this model using human cardiac adipose tissue from heart surgeries, significantly increasing its translational potential. These human adipoids are functional- building monocular lipid droplets and generating immune cells. Translating to humans gives many opportunities for research on adipose tissue-related diseases and opens up the possibility of using adipoids for personalised medicine.

Presentation: Poster

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Application of advanced *in vitro* models for mechanistic translational understanding of cardiovascular liabilities

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Cardiovascular safety findings encompass a range of perturbations covering ECG changes, haemodynamics and cardiac pathology, which can occur independently or concomitantly. Due to the complex and often chronic nature of drug-induced toxicities, there is often a reliance on *in vivo* studies to predict structural pathologies in preclinical drug development. Monoculture *in vitro* assays have greatly reduced attrition in early drug safety screening and are invaluable for the prediction of hERG and contractility risks, for example. However, they do not offer the architectural and multicellular complexity akin to the *in vivo* environment, limiting toxicological predictivity and mechanistic insight gleaned from these assays. Incorporation of relevant cell types and complex orientations are crucial to recapitulate the human *in vivo* tissue situation.

Here we will discuss 2 case studies which exemplify the need for multicellular *in vitro* assays alongside novel technology to extract mechanistic insight from toxicological responses.

Firstly, we have developed a 3D multicellular cardiac microtissue model that offers increased physiological relevance in a format amenable to drug discovery. We have successfully developed *in situ* single cell signalling pathway analysis (TOBis IMC) which integrates imaging mass cytometry (IMC) with a customised barcoding strategy of thiol-reactive organoid barcoding *in situ* (TOBis). The *in situ* single cell signalling data allows us to identify different patterns of cardiac cell-type specific responses to cardiotoxin treatments. This approach could be used as the basis for further mechanistic understanding of structural cardiotoxins.

Furthermore, drug-induced vascular toxicities are challenging to predict early on in drug discovery. Our aim was to utilise a multicellular vessel-on-a-chip to capable of revealing novel cell signalling interactions in response to agents causing vascular perturbations. This was facilitated via utilisation of the Emulate organ-on-a-chip technology enabling both human endothelial cells and smooth muscle cells isolated from a major vasculature bed and cultured in two flow channels separated via a semi-permeable membrane, optimal flow was applied to enable the key physiological features of a vessel to be mimicked.

The development of advanced physiologically relevant cardiovascular *in vitro* models and associated technology are paving the way for the deeper mechanistic understanding of drug-induced cardiovascular liabilities.

Presentation: Poster

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Mathematical modelling combined with microphysiological systems (MPSs) enables the quantitative assessment of clinical safety in early stages of drug development

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Modern drug safety assessment strategies aim to integrate *in-vitro* experimental methods and modelling approaches to increase patient safety while reducing drug development costs. Translational strategies require the integration of the drug effect, quantified in *in-vitro* settings, into modelling frameworks suitable to describe the dynamic interaction of multiple organic processes in patients responding to the drug challenge.

We have developed quantitative systems models, focussed on the assessment of oncotherapeutics safety, in an iterative refinement fashion between preclinical and clinical investigative toxicology. We are presenting our suite of multiscale models of the intestinal epithelium to predict the dynamics of drug-induced injury and clinical diarrhea, based on both the drug mechanism of action and the toxicity measured in *ex-vivo* grown epithelial organoids. A

comparative analysis of the observed and predicted clinical diarrhea incidence for several compounds has demonstrated that this modelling strategy leads to superior predictive performance when compared against *in-vivo* based predictions. Similarly, our bone marrow MPS-modelling framework is routinely applied to predict the clinical haematological landscape associated with oncology compounds prior to clinical trials and several published clinical studies have been used to assess the performance of our MPS-based haematotoxicity model to predict clinical cytopenia risk.

Altogether, we demonstrate that the integration of *in-vitro* advanced MPSs and mathematical models enables the quantitative safety assessment of investigational drugs at early stages of the development pipeline to support decision making and clinical trial design.

Presentation: Oral

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Modeling the stages of cervical cancer pathogenesis: Establishment of a healthy cervix-, a pre-cancerous CIN- and an immunocompetent carcinoma-on-chip

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Introduction: The sexually transmitted Human Papilloma Virus (HPV) leads to infections in most sexually active people at least once in their lifetime. While most (asymptomatic) infections of the female *C*erviX (*CX*) clear themselves, upon persistent infection a pre-cancerous *C*ervical *I*ntraepithelial *N*eoplasia (*CIN*) can develop and progress to a *C*ervical *C*ancer (*CC*). CC is the 4th most common cancer within women, leading to > 340,000 deaths worldwide and to a huge need for immunotherapeutic options. Hence, human-based, immunocompetent physiological *in vitro* models of cervical carcinoma pathogenesis are urgently required to gain knowledge in basic research and for the development of (novel) therapeutic options.

Methods: A tailored microfluidic platform is fabricated by combining layers of laser-cut and hot-embossed thermoplastics and thermoplastic elastomers. Keratinocytes, fibroblasts, and neutrophils are isolated from human cervical tissue biopsies and whole blood of healthy donors. Depending on the model, primary cells as well as the cervical squamous cancer cell line SiHa are combined in dynamic co- or triple-cultures in a hydrogel or scaffold to emulate the 3D architecture. Neutrophils are perfused through the channel. The tissues are characterized via on-chip immunofluorescent staining and effluent analysis as well as off-chip histology.

Results: The developed MPS incorporates two independent systems with four replicate tissue chambers each, allowing air-lifted as well as submersed cultivation of tissue. The healthy tissue in the CX-on-chip is characterized by a 3D stromal layer with primary fibroblasts and on top of it a multi-layered keratinocyte epithelium expressing signs of differentiation. The CIN-tissue with a SiHa-derived epithelium does not differentiate and expresses the proliferation marker Ki67 in all layers. In the CC-on-Chip, SiHa spheroids emulate cancerous nests that respond to co-culture with fibroblasts and to compound treatment. Neutrophils can be perfused through the blood-vessel mimicking channel and migrate into the cancerous tissue.

Conclusion: We established a novel design and fabrication concept for a hybrid-material MPS that allows the generation of physiological human micro-tissues of three different stages in the development of cervical cancer. Further integration of immune components in these platforms will boost research in immune-oncology therapy [1].

Reference

[1] Kromidas, Maulana et al. (2021). *Adv Drug Deliv Rev*.

Presentation: Oral

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Tissue chips in space: Modeling human disease states in microgravity

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Several biological systems weaken or deteriorate in microgravity – cardiac function, musculoskeletal mass, bone density, visual acuity, and the immune system – and these dysfunctions closely mirror some age-related disease states. These microgravity-induced phenomena provide the opportunity to model these diseases in space and gain insights into the mechanisms controlling age-related dysfunction. Through partnerships between NCATS, NASA and the Center for Advancement of Science in Space, the Tissue Chips in Space program was created (https://ncats.nih.gov/tissue-chip/projects/space) to study the effects of microgravity environment on the human body at the International Space Station National Laboratory. The Tissue Chips in Space program supports development of tissue chip and organ-on-a-chip platforms that model physiological changes associated with aging and related diseases. This program enabled advances in the study of microgravity-associated age-related conditions mimicking accelerated aging pathophysiology in a relatively shorter period of time than it would take

to undertake the same studies on earth. We have learned how microgravity exerts a unique range of stresses and pathophysiological perturbations on the human body resulting in dramatic increase in oxidative stress and inflammation, muscle wasting, immune senescence, cardiovascular deconditioning and cardiomyopathy, alteration of gene expression and DNA damage. These findings have expanded our understanding of age-related conditions and will contribute to drug development that can slow the process of aging and lead to new interventions to improve human health. This program has also made key technological improvements in the tissue chips instrumentation systems towards automation and miniaturization required for space flight. These technological advances will be used for future engineering of tissue chip platforms for ease of use and broader accessibility of tissue chips in drug development and biomedical research here on Earth.

Presentation: Oral

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Connecting organs, a standardized approach to multi-organ chip connections

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Organ-on-chip (OOC) platforms recapitulate the microenvironment for a given tissue as an alternative to traditional drug research. Most systems focus on single tissues, suitable for initial proof-of-concept studies, however, these are lacking communication between the different body tissues. This is essential especially in drug development, immunotherapies, or cancer research. Another limitation of current OOC development, is the lack of universal chip standards, leading to uniquely shaped systems and difficult integration into pre-existing equipment. Here, we present the development of a multi-chip platform allowing different OOC systems to be connected and being perfused by circulating immune cells. This platform considers the requirements presented by industry players in this field [1].

The design accommodates footprints of common laboratory read-out equipment that is the standardized glass slide footprint or standardized multi-well plates. The individual chip design follows the ISO standards in terms of margins and port dimensions [1]. Besides the organ chips, there are additional specialized chips which provide other functionalities such as secure connections to tubing and to organ modules, and at a later point reservoirs and on-chip pumps. The chips are connected through spring-loaded fasteners and are sealed by the choice of elastomer material. Initial characterization showed capability of handling flow rates up to 350 µL/h.

Importantly, the multi-organ setup allowed circulation of immune cells for 24 h without prominent activation. Long-term T-cell perfusion was performed for 72 h which will be followed by further analyzation of system-induced changes though FACS analysis. Future module-integrations aim to recreate immunological mechanisms of tumor growth and tumor-lymph node interaction. Besides, a specifically tailored immune cell reservoirs was developed which keeps circulating immune cells in suspension providing physiological perfusion support.

This approach shows promise for future integration of mature organ systems, their complex (immune-)interactions, and sophisticated drug studies. Standardization and compatibility concepts facilitate the acceptance by (end-)users.

The authors wish to thank the Wellcome LEAP foundation for funding and support through the scope of the Human Organs, Physiology and Engineering (HOPE) program.

Reference

[1] International Organization for Standardization, Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification, ISO 22916:2022.

Presentation: Poster

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Towards a proximal tubule microphysiological system for antisense oligonucleotide safety testing

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Antisense oligonucleotides (ASOs) enable the modulation of clinically relevant targets that cannot be accessed with other therapeutics by adjusting the expression of the target mRNA. However, ASO accumulation in kidney proximal tubules is a well-known property that can become a safety liability for less tolerated compounds. The PCSK9-targeting ASO SPC5001 was terminated in a clinical phase 1 study due to dose-related tubulotoxicity [1], which the preceding preclinical toxicity studies performed in mice and non-human primates failed to predict. This drug-induced kidney injury (DIKI) has subsequently been detected using a proximal tubule microphysiological system (MPS) [2]. In the current study, we evaluated whether ASO-related target gene knockdown and tubulotoxicity could also be observed using the HUMIMIC Chip4, an MPS that enables the long-term co-cultivation of a proximal tubule model with a glomerulus model, endothelial cells and up to three

additional organ models of interest. This constitutes a highly physiologically relevant model that can be used to assess organ-specific toxicity, targeting and exposure routes in future studies.

Here, we seeded primary hRPTECs into the HUMIMIC Chip4 and cultivated them using a defined fluid flow and shear stress for 28 days without the addition of further organ models. HRPTECs maintained a confluent monolayer and exhibited their typical morphology for the entire cultivation period within the chip and the static control well plates. Following a 7-day equilibration phase, cells were repeatedly exposed to the nephrotoxic ASO SPC5001 or a control ASO [3] with substance application on days 7, 14 and 21, mimicking a weekly clinical dosing regimen. SPC5001 treatment resulted in an efficient target gene knockdown in dynamic and static cultures with a 10-fold lower PCSK9 expression on day 28 compared to the controls, as well as increased lactate secretion and an increased number of dead cells.

In conclusion, this proximal tubule MPS presents a promising tool for future ASO toxicity studies with the possibility of extending the cultivation and exposure period, incorporating additional organ models or utilizing PBPK modelling and *in vitro-in vivo* extrapolation to enable clinically relevant predictions of ASO toxicity.

References

[1] doi:10.1053/j.ajkd.2013.02.359
[2] doi:10.1007/s00204-021-03062-8
[3] doi:10.1016/j.omtn.2016.11.006

Presentation: Poster

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Evaluation of photobiomodulation effects on vascular network using physiological model and vascular network on chip

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Photobiomodulation (PBM) in near infrared could improve viability and functionality of many cell types in protecting them from external stress (hypoxia, anorexia, cytokinic stress…). In the context of diabetes and islet transplantation, we hypothesize that PBM could have a positive impact on the transplanted islets of Langerhans but also on the vascular network of the patient which are stressed during transplantation.

To study at first the impact of PBM on vascular network *in vitro* without any stress, a solution is to illuminate a vascular network model and characterise the development and stability of the network with and without illumination.

Here, the objective is to evaluate the impact of PBM on an *in vitro* 3D vascular network made with human fibroblasts and endothelial cells in a fibrin hydrogel. The goal is to determine whether PBM can have a quantifiable effect on the network, and if so, whether it is a positive or negative effect.

Positive effect will allow vascular growth and ensure stability and longevity of the existing network. Neutral effect will not affect the vascular network in terms of growth and stability. Finally, negative effect will decrease the survival and longevity of the network.

A specific illumination bench is used that has been designed and developed to be able to illuminate samples directly in the incubator. Previous work has demonstrated that long illumination time at moderate power were efficient on 2D cells culture to preserve cells viability during an external stress.

We demonstrate that PBM is not deleterious for the vascular network. Moreover, applying this long illumination time of several hours on 3D vascular network seems to have a slight positive effect.

In addition to morphological characterizations, secretions of cells are also measured to access characterizations that are more functional.

The impact is assessed first on 3D static vascular model and will be then evaluated on 3D fluidic model to be closer from physiological conditions.

This work is a first step on studying PBM effects and will be completed with a model that is more physiological thanks to OOC.

Presentation: Poster

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A new human dynamic integrated organ (MPS) platform for developing *in vitro* pharmacokinetic and toxicity data

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The development of an integrated organ MPS system that can be used to identify pharmacokinetic parameters and organ toxicity is important for the development of non-animal methods and risk assessment in the future. The purpose of this study was to evaluate chemicals that the US FDA has determined to be of interest in a new integrated organ platform. The test compounds were Acrylamide and Usnic acid. Studies were done to understand non-specific adsorption, flow, dose selection, and analytical method op-

timization prior to the full experiment. The complete test circuit consisted of a human 3D EpiIntestinal®, human primary hepatocytes and human kidney cells (HK-2). Organ compartments were linked via a simulated blood system. Communication between organs occurred via osmotic flow through semi-permeable membranes. Simulated blood flow was achieved with a micro syringe pump. Acrylamide (0.3, 0.5, 1.0 mg) and Usnic acid (6, 20, 60 mg) were applied (0.1 mL) to the apical side of the intestinal chamber and samples were collected from all organ compartments at multiple times (0.5, 1, 2, 5, 10, 24, 48, and 72 h). Simulated blood contained saline with human serum albumin (0.4%). Cytotoxicity was monitored by LDH, ATP, GSH and NAG at 72 h. The resulting kinetic curves showed excellent dose-response relationships (Cmax for Acrylamide 200, 100, 50 µg/mL and Cmax Usnic 20, 9, 2.5 µg/mL) there were clear absorption and elimination components to the concentration profiles in each organ chamber. For Acrylamide the liver and kidney showed the highest toxicity, and for Usnic acid the liver showed highest toxicity. These toxicity findings are in agreement with *in vivo* data in the published literature.

Presentation: Oral

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Human 3D InSight™ liver spheroids are a highly predictive *in vitro* model for predictive and investigative toxicology

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Hepatotoxicity is an important safety hazard that can lead to the discontinuation of the development of new drug candidates. Micro-physiological systems (MPS) raised expectations that their use in industrial practice would improve hepatoxicity assessment. Among the available MPS, Human 3D InSight™ Liver spheroids accurately model essential hepatic features and predict hepatotoxicity more accurately than 2D hepatocyte cultures [1]. To further evaluate the relevance of these liver spheroids in hepatotoxicity assessment, the cytotoxicity of 82 small molecule drugs selected from the DILIrank dataset was evaluated by measuring their 7-day cellular ATP IC50 value in the liver spheroids. The DILIrank dataset consisting of 1,036 FDA-approved drugs is divided into four classes according to their potential for hepatotoxicity: "Most-DILI-concern", "Less-DILI-concern", "No-DILI-concern" and "Ambiguous-DILI-concern". When normalizing the *in vitro* cytotoxicity data with human exposure concentrations (total plasma Cmax), 80.6% of "Most-DILI-Concern" drugs were accurately predicted as hepatotoxic, and 84.2% of "No-DILI-Concern" drugs were accurately predicted as non-hepatotoxic.

Moreover, the physiological relevance of the 3D InSight™ liver spheroids make it an effective *in vitro* tool for studying toxicological mechanisms of clinical hepatotoxicants. Using different treatments of the liver spheroids, the *in vivo* mechanisms of toxicity of aflatoxin B1 and trovafloxacin were evaluated. Trovafloxacin stabilizes TNF-α signalling, resulting in liver injury in otherwise benign liver inflammatory conditions. Correspondingly, trovafloxacin cytotoxicity was enhanced in the liver spheroids treated with lipopolysaccharides, suggesting that the *in vivo* synergic interplay between trovafloxacin and inflammation is recapitulated *in vitro*. Aflatoxin B1 is activated *in vivo* by CYP3A4 and CYP1A2. Correspondingly, the pan-specific CYP450 inhibitor 1-aminobenzotriazole decreased aflatoxin B1-mediated cytotoxicity in the liver spheroids, suggesting that the *in vivo* activation of aflatoxin B1 by CYP450 enzymes is recapitulated *in vitro*.

The sensitivity, specificity, easy-of-use, and cost-effectiveness the 3D InSight™ liver spheroids make them a productive and industry-compatible MPS for liver safety assessment. Moreover, their scalability offers the opportunity for *in vitro* high throughput screening for specific *in vivo* mechanisms of hepatotoxicity and enables the generation of high-quality hepatotoxicity datasets which can be supportive for the critical go/no-go decision-making during the drug development process.

Reference

[1] Proctor et al. (2017). *Arch Toxicol*.

Presentation: Oral

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Melt electrowriting and freeform printing for biofabrication of *in vitro* vascularization

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Functional 3D tissue models are a potentially valuable tool to provide more transferable test results regarding basic research, drug development or other applications than classic 2D cell culture. However, the biggest bottleneck in the generation of functional 3D tissue models is the incorporation of a reproducible and perfusable vascularization, mimicking the *in vivo* hierarchy accurately and keeping bigger 3D tissue models alive. In this study, we investigated the potential of Melt Electrowriting (MEW) [1] and Freeform

Printing (FFP) [2] to generate complex microfiber networks, which can be dissolved on demand in crosslinkable hydrogel matrices for the generation of accurate and perfusable microchannel networks.

For this, we introduced biocompatible poly(2-oxazoline)s as a material for sacrificial scaffolds. These polymers are thermoresponsive in aqueous solutions, meaning that they dissolve on demand by simple temperature reduction. We demonstrated that poly(2-oxazoline)s can be fabricated via the modern additive manufacturing technologies MEW and FFP to generate highly accurate microfiber networks in 2D and 3D. In aqueous environments, e.g., upon embedding in hydrogels, the fibers swelled, leading to fusion of adjacent filaments. Subsequent lowering of the temperature led to scaffold dissolution on-demand, without leaving non-dissolvable remnants and resulting in a leakage-free connection to the medium reservoirs. This approach enabled the generation of interconnected channel networks with bifurcations according to Murray's law, resembling the natural vascularization. Importantly, the developed method is compliant with many of currently used hydrogels (e.g., methacrylated gelatin or collagen), suitable for vascularized tissue engineering approaches. Furthermore, the scaffolds with more complex 3D geometries could be produced using poly(2-oxazoline)s in combination with FFP method. This opens multiple possibilities in creating viable 3D constructs of larger dimensions, containing microvascular network to support cell survival and tissue maturation.

References

[1] Ryma, M., Genç, H., Nadernezhad, A. et al. (2022). A print-and-fuse strategy for sacrificial filaments enables biomimetically structured perfusable microvascular networks with functional endothelium inside 3D hydrogels. *Adv Mater 34*, 2200653.

[2] Mair, V. et al. (2022). Freeform printing of thermoresponsive poly(2-cyclopropyl-oxazoline) as cytocompatible and on-demand dissolving template of hollow channel networks in cell-laden hydrogels. *Biofabrication 14*.

Presentation: Oral

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Virotherapy *in vitro* – development of an organ-on-chip model for treatment of tumoroids with oncolytic viruses and chemotherapy

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Cancer treatment with oncolytic viruses (OVs), which can replicate selectively in tumor cells, is a new approach in immunotherapy. The efficacy of these drugs cannot be accurately translated from animals to humans, highlighting the need for complex *in vitro* models. Replicating the treatment of patients requires simulation of blood flow and the tumor in its tumor microenvironment (TME). The blood contains various inhibitory elements such as neutralizing antibodies (nABs) and adds physical factors like shear forces. The tumor model does not only require a 3D structure, but also the TME with immune cells, extracellular matrix, and vasculature [1].

Focusing on colorectal and lung cancer, spheroids containing cancer cell lines and cancer-associated fibroblasts are generated in ultralow attachment plates allowing matrix-free 3D culture. This allows direct treatment of the spheroids with OVs, modified with a fluorescent transgene to monitor infection, or chemotherapy. We have shown that the spheroids are sensitive to chemotherapy and viral infection, which can be inhibited by nABs in a concentration-dependent manner. In addition to treatment in static conditions, an organ-on-chip model is currently being developed to better mimic the highly dynamic conditions in the body. The microfluidic chip consists of a tumor chamber connected to a channel coated with endothelial cells, recreating vascular and tumoral compartments. This allows mimicking blood flow and intravenous injection of treatments. Currently, endothelial cells of different origins are being tested to achieve a stable endothelial barrier. Initial experiments showed infection of the spheroid in the tumor chamber and the endothelial cells after virus perfusion. The next step is to add a vascular network to the tumor chamber.

The development of a complex model that incorporates the TME and the endothelial barrier will allow us to study the efficacy of OVs. In a next step, patient derived organoids will be used to replace the spheroids to study the patient-dependent heterogenous response to OVs or chemotherapy as well as the development of resistance to these treatments. To increase the potential of the model for use in medical research, elements related to the immune and circulatory systems will be added.

Reference

[1] Martinez-Quintanilla, J. (2019). *J Clin Invest*.

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Differentiation of iPSCs into neocortical neurons in a microphysiological environment

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Induced pluripotent stem cells (iPSC)-derived neural 2D cultures have been used to better understand cellular and molecular mechanisms underlying human neocortical development but lack the

complexity of native tissue. Cerebral organoids, 3D models of human neocortical development, could address this caveat, but to date still have only limited capacity to recapitulate human neocortical development and are hampered by high variability. Hence, it is critical to further develop cerebral models in a microphysiological environment by improving the spatiotemporal unfolding of their anatomical organization, enhancing reproducibility, and making them more accessible for pharmacological interventions.

We have demonstrated that iPSCs can be differentiated into neocortical neurons in a microphysiological environment.

First, parameters of iPSCs differentiation, such as cell seeding density, extracellular matrix (ECM) type (Matrigel, Collagen, Polyvinyl alcohol (PVA)-HA and HyStem-C gel) and duration of differentiation, were screened in standard 96-well plates. Cells seeded into PVA-HA and HyStem-C at higher density demonstrated better neural differentiation.

To demonstrate iPSCs embedded in PVA-HA and HyStem-C hydrogel could survive, proliferate, and differentiate on chip, we tested cell differentiation in OrganoPlate® 3-lane 40. Each chip of OrganoPlate 3-lane 40 platform contains one cell culture channel and two (perfusion) channels. We seeded the cell-gel mixture into middle channel within 2 perfused channels. From on-chip differentiation, 3D networks of neurons were observed. Importantly, immunostaining indicates that cells were differentiated toward deep layer neocortical fate analogously to differentiation in conventional organoid protocols.

In conclusion, our study reveals the possibility of differentiating iPSCs into neuronal networks. Custom-made microfluidic chip will be designed to establish morphogen gradients which will improve layering of different cell types.

Presentation: Poster

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Bioengineered 3D cardiac tissue model for cardiotoxicity studies

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Bioengineered 3D cardiac tissue models have the potential to revolutionize the way chemical toxicity is assessed. These models are often engineered using human-derived cells, such as stem cells, which are differentiated into heart muscle cells and assembled into a three-dimensional structure. Integrating different cell typologies (endothelial cells, fibroblasts) improves stem cell-derived cardiomyocyte maturation [1].

Bioengineered 3D cardiac tissue models could mimic a human heart's spatial and cellular organization, providing a more accurate and reliable tool for testing the effects of chemicals on the heart.

Our work compares the result of a co-culture of hiPSC-CMs (80%) and HCAECs (20%) embedded in a 3D gel network based on photo-crosslinked gelatin methacryloyl (GelMA) with respect to the gold-standard 2D hiPSC-CMs culture treated with Doxorubicin 2uM. GelMA was synthesized with a 100% degree of methacryloylation, sterilized with EtO, solubilized in a cell culture medium, and photo-crosslinked upon cell encapsulation at 365 nm in the presence of a catalytic amount of photo-initiator. Chemical and rheological characterization was performed to assess synthesis success and investigate hydrogel properties.

Cells were cultured for 7 days and then treated for another 7 days with Doxorubicin. At the end of 14 days, several cell functions (mitochondrial function, oxidative stress, cell integrity, cardiac, and specific cell markers) were analyzed to observe their modulation in the different *in vitro* systems.

The results show, in the 3D culture, an increase in mitochondrial function and a reduction of the release of ROS, LDH, and high-sensitive Troponin I, providing evidence of a more resistant phenotype of the bioengineered model than the 2D system, confirming data of literature [2].

This work was supported by the European Union's Horizon 2020 research and innovation program (grant #101037090). The content of this manuscript reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains

References

[1] Dunn, K. K. et al. (2019). *Biotechnol J 14*, e1800725.
[2] Edmondson, R. et al. (2014). *Assay Drug Dev Technol 12*, 207-218.

Presentation: Oral

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Neurodegenerative and rare diseases investigations utilizing human-on-a-chip systems

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We have been constructing multi-organ human-on-a-chip systems for toxicology and efficacy with up to 6 organs and have demonstrated long-term (> 28 days) evaluation of drugs and compounds, that have shown similar response to results seen from clinical data or reports in the literature. Application of these systems for neurodegenerative and rare diseases such as ALS, Alzheimer's, chron-

ic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), and Myasthenia gravis (MG) will be described. These models utilize a pumpless platform with serum free recirculating medium, which is a low volume system that can evaluate parent compounds as well as metabolites, if the liver is included. Our research focus is on the establishment of functional *in vitro* systems to address phenotypic deficits to create organs and subsystems to model motor control, muscle function, myelination and cognitive function, as well as the potential for including cardiac, BBB, kidney, GI tract and liver subsystems. Acute and chronic compound testing in systems for concurrent measurement of both efficacy and toxicity has also been done in the same system for therapeutic index estimation. A specific embodiment of this technology is the creation of a functional human NMJ system to understand ALS and MG. We have investigated the four primary mutations found in ALS patients; SOD1, FUS, TDP43 and C9ORF72 and demonstrated variations of the disease phenotype as well as response to therapeutics with pharmaceutical companies. MG patient sera samples have also been investigated in the NMJ platform. We also will describe an Alzheimer's disease model based on long-term potentiation, a correlate for learning and memory, which has reproduced aspects of amyloidopathy and tauopathy, and shown drug selective reversal with current AD therapeutics. Sanofi has used efficacy data from our conduction velocity model to file an IND for CIDP that has enabled a clinical trial (#NCT04658472) and is described in our recent joint publication. We will also describe a multi-organ innate immune system that was able to reproduce the pro-inflammatory and restorative phenotypes from macrophages.

Presentation: Oral

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Establishment of a dynamic *in vitro* human iPSC-derived blood-brain barrier for investigating the passage of biologics

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The effectiveness of biologics for the treatment of brain disorders is still constrained by the blood-brain barrier (BBB). Current delivery approaches, such as injection into the cerebrospinal fluid, are invasive and the amount of carried biologic might not be sufficient to elicit a therapeutic response [1]. Receptor-mediated transcytosis (RMT) is a suitable alternative to increase brain exposure to biologics. Indeed, antibodies targeting receptors, such as transferrin receptor, on the plasma membrane of brain microvascular endothelial cells (BMEC) can serve as shuttles to improve the passage of biologics after systemic administration [2]. However, antibody properties influence their interaction with receptors and their intracellular fate, affecting how efficiently they can be transcytosed to reach the target site [3].

In this perspective, *in vitro* BBB models that accurately resemble normal physiology and disease pathophysiology are essential for target validation and for providing insight into the permeability of biologics across the BBB. The combination of microphysiological systems with advances in induced pluripotent stem cells (iPSC)-derived BMEC has the potential to address this request by offering a predictive BBB model.

Therefore, we used the OrganoPlate® 3-lane system (Mimetas) to create a perfused human *in vitro* BBB model made of iPSC-derived BMEC for investigating the passage of biologics. The established model showed physiological transendothelial electrical resistance, strict paracellular tightness to fluorescence tracers, and expression of key BBB markers such as tight junction proteins, transporters and receptors. The barrier was functional for P-glycoprotein activity and transferrin receptor-mediated transport. Moreover, the model enabled the selective shuttling of antibodies through the BBB.

Our findings clearly show the suitability of iPSC-derived BMEC cultured in the Mimetas platform for the evaluation of antibody receptor-mediated transcytosis, a requirement for brain shuttle technology.

References

[1] Pardridge, W. M. (2020). Blood-brain barrier and delivery of protein and gene therapeutics to brain. *Front Aging Neurosci 11*, 373.

[2] Niewoehner, J. et al. (2014). Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron 81*, 49-60.

[3] Sade, H. et al. (2014). A human blood-brain barrier transcytosis assay reveals antibody transcytosis influenced by pH-dependent receptor binding. *PLoS One 9*, e96340.

Presentation: Poster

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A human kidney and liver organoid-based multi-organ-on-a-chip model to study the therapeutic effects and biodistribution of mesenchymal stromal cell-derived extracellular vesicles

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Mesenchymal stromal cell (MSC)-derived small extracellular vesicles (sEVs) show therapeutic potential in multiple disease models, including kidney injury. Clinical translation of sEVs requires further preclinical and regulatory developments, including elucidation of the biodistribution and mode of action (MoA). Biodistribution can be determined using labelled sEVs in animal models which come with ethical concerns, are time-consuming and expensive, and may not well represent human physiology. We hypothesized that, based on developments in microfluidics and human organoid technology, *in vitro* multi-organ-on-a-chip (MOC) models allow us to study effects of sEVs in modelled human organs like kidney and liver in a semi-systemic manner.

Human kidney- and liver organoids combined by microfluidic channels maintained physiological functions, and a kidney injury model was established using hydrogen peroxide. MSC-sEVs were isolated, and their size, density and potential contamination were analyzed. These sEVs stimulated recovery of the renal epithelium after injury. Microscopic analysis shows increased accumulation of PKH67 labelled sEVs not only in injured kidney cells but also in the unharmed liver organoids compared to healthy control conditions.

In conclusion, this new MOC model recapitulates therapeutic efficacy and biodistribution of MSC-sEVs as observed in animal models. Its human background allows for in-depth analysis of the MoA and identification of potential side effects.

Presentation: Oral

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Cryo(bio)printing for anisotropic tissue manufacturing

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Bioprinting is an innovative research method. In our latest study, we blended cryoprotectants with bio-inks to develop a new bio-ink. This bio-ink can be bioprinted at a low temperature from zero to minus 20 degrees Celsius, and the printed scaffold can be stored at a low temperature before application. The study found that the cryopreserved scaffold has biological activity and can realize osteogenic differentiation and angiogenesis. And we freeze-cast this bio-ink to realize vertical bio-3D printing. And we have achieved coaxial bioprinting and multi-material bioprinting using this method. In addition, we found anisotropic channels in the scaffold, and we realized the anisotropic distribution of cells in the scaffold by using this channel. We used this characteristic to implant bone marrow mesenchymal stem cells, myoblasts, tenocytes, vascular endothelial cells, etc. into them and found that it can induce the anisotropy of cells forming tissues. Using this characteristic, we have carried out muscle tissue engineering, and realized the construction of muscle tendon microunits and muscle blood vessel microunits. This method can be effectively used in tissue engineering, drug screening, tissue models, personalized therapy, etc.

Presentation: Poster

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Assessment of commercial drug compounds in an engineered heart tissue platform using human induced pluripotent stem cell-derived cardiomyocytes in serum-free media

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Adverse drug reactions during the preclinical and post-approval phase due to cardiotoxicity effects, combined with the drug development times and costs, highlight the critical need for human-based translational models for assessing the contractility effect of new drug compounds during preclinical target validation and lead optimization [1]. Currently, engineered heart tissues (EHTs), generated from human pluripotent stem cell-derived cardiomyocytes, have

shown the advantage of allowing the assessment of contractile performance in a three-dimensional configuration that resembles the *in vivo* situation [2]. By using an EHT platform [3], we evaluated the suitability to detect drug-induced changes in the contractility of hiPSC-EHTs by 20 commercially available drug compounds with different inotropic effects (positive, negative) or no-effect. Additionally, all the testing of the drug compounds were done using serum-free media to avoid protein-drug binding, that might affect the response of the EHTs to the compounds. Contractile performance was evaluated by analyzing the amplitude, velocity and duration of contraction and relaxation. In 80% of the cases, we were able to observe the expected drug response. In addition, for most of the tested compounds a dose-response relationship was found. These results show that this human-based cardiac *in vitro* model using serum-free media is a suitable tool to assess the effect of drug compounds on the contractile performance of cardiomyocytes and as such can be used in the early drug discovery and development process.

References

[1] Savoji, H., Mohammadi, M. H., Rafatian, N. et al. (2019). Cardiovascular disease models: A game changing paradigm in drug discovery and screening. *Biomaterials 198*, 3-26. doi: 10.1016/J.biomaterials.2018.09.036

[2] Stein, J. M., Mummery, C. L. and Bellin, M. (2021). Engineered models of the human heart: Directions and challenges. *Stem Cell Reports 16*, 2049-2057. doi:10.1016/J.STEMCR.2020.11.013

[3] Ribeiro, M. C., Rivera-Arbeláez, J. M., Cofiño-Fabres, C. et al. (2022). A new versatile platform for assessment of improved cardiac performance in human-engineered heart tissues. *J Pers Med 12*, 214. doi:10.3390/JPM12020214

Presentation: Oral

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Tonsil-on-chip to test T cell-dependent antibody responses and vaccine efficacy *in vitro*

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Vaccinations against viral and bacterial infections are the best strategy to control endemic and epidemic diseases and to prevent potential related long-term impact on human health. Although vaccine development has made fast progress in the past decades, many vaccines fail in advanced stages of development and clinical trials. One reason for late-stage failures is related to the poor predictive value of pre-clinically applied *in vivo* and *in vitro* methods due to species-specific differences in the immune system and insufficient reflection of physiological vaccine responses respectively.

We have developed a Tonsil-on-Chip system which allowed culture of primary tonsil cells in a tissue chamber under perfusion for at least two weeks. The tissue chamber of the microfluidic platform was designed to enable high density seeding of tonsil cells and was separated from the medium channel by a semi-permeable membrane. The medium channel of tonsil cell-loaded chips was perfused either with vaccine-free medium, vaccine-supplemented medium or medium containing antigen-presenting cells pre-incubated with vaccines. Immunofluorescence staining, cell tracker labelling and confocal imaging was used to observe differences in cell distribution and abundancy inside the chamber as well as trans-membrane migration of antigen-presenting cells. Repeated sampling of effluents allowed time-resolved analysis of cell viability, cytokine release and activation status of immune cells.

Using our Tonsil-on-Chip system, we observed successful trans-membrane recruitment of antigen-presenting cells into tonsil tissue and time-resolved production of cytokines and antibodies. The Tonsil-on-Chip system can be used to evaluate differences in immune responses to existing and newly developed vaccines depended on the mode of antigen delivery by comparing the germinal center reactions inside the tonsil tissue, the quality of antibody responses and the level of cytokine production in perfused medium. In addition, the system can be used to determine the efficacy of vaccine candidates in a more physiological manner.

Presentation: Oral

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A deep-learning-assisted image analysis and a multiparametric biochemical quantification in human 3D model of non-alcoholic steatohepatitis for high-throughput drug discovery

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Non-alcoholic steatohepatitis (NASH) is a progressive severe disease characterized by lipid accumulation, inflammation, and fibrosis in the liver which lacks any approved drug therapy. Novel approaches to identify therapeutic candidates that predict clinical responses are needed. Human pre-clinical models, including organoids/spheroids, are powerful for translational drug discovery, however, the use of high-throughput scalable methods including drug screening is a current challenge.

We have previously developed a human-derived 3D spheroid model, containing primary human hepatocytes, Kupffer cells, liver endothelial cells, and stellate cells. By inducing a NASH-inducing cocktail we were able to recapitulate the main hallmarks of NASH including steatosis, inflammation, and fibrosis. We show here robust endpoints amenable for high-throughput drug screening aiming at a multiparametric phenotypic approach. Intracellular triglycerides, and secretion of pro-collagen I, as surrogate markers of steatosis and fibrosis respectively were normally distributed (Kolmogorov-Smirnov test) and showed a Z' score above 0.5. Both triglycerides and pro-collagen I analysis were performed from the same individual spheroids (n = 40).

To complement these biochemical and soluble endpoints, we developed an imaging approach to quantify total lipids and lipid droplets features including size and intensity. We made use of a high throughput spinning disc confocal instrument to capture optical sections through approximately 70 µm of the microtissue sample. Much like human clinical samples, the lipid droplets in the disease-induced microtissues were present at extremely high densities and varied considerably in size (from < 1 µm to > 50 µm in diameter) and fluorescent intensity. To circumvent the time-consuming and often poor segmentation of densely packed objects by classical image analysis, we employed an algorithm powered by deep learning to precisely detect micro and macro-steatosis, as well as dim and bright lipid droplets in the 3D NASH model.

The resulting workflow will serve as the foundation for a "first-of-its-kind" drug discovery screen utilizing a deep learning approach to analyse the 3D image files derived from a human microtissue steatosis model. In combination with biochemical endpoints, it represents the path for affordable and predictable drug discovery in NASH using human *in-vitro* 3D models.

Presentation: Poster

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Rapid 3D-bioprinting approaches for studying human vascular disorders

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Organs-on-Chips (OOCs) and Microphysiological Systems (MPSs) hold great promise as a more ethical and effective alternative to traditional animal testing. However, previous OOCs and MPSs have been limited by the drawbacks of conventional microfabrication methods and materials including absorption of small molecules and limited scaling. In my talk, I will describe how our laboratory is using a hybrid 3D bioprinting approach that combines Stereolithography (SLA) and Extrusion bioprinting to rapidly prototype functional Microphysiological Systems (MPSs) made of multiple human cell types and a microfluidic hydrogel. This approach provides a more natural 3D environment for cells, enabling them to recreate higher-order tissue structures and respond to fluid flow and clinically relevant drugs. Our bioprinted model of human glioblastoma highlights the importance of interstitial fluid flow and the presence of brain endothelial cells and astrocytes in stimulating the expression of cancer stem cell markers, such as CD133. This model offers a unique platform for testing brain cancer chemotherapeutics, monitoring drug effects on normal tissues, and developing novel therapies targeting the microenvironment. We have also developed a 3D bioprinted model of blood clotting to study the adverse events caused by the Spike protein during severe COVID-19 infection. Our research demonstrates the versatility of our hybrid 3D bioprinting approach and holds the potential for advancing our understanding of the molecular mechanisms underpinning blood clotting in various diseases and contributing to the development of new therapies.

Presentation: Oral

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From developmental biology to drug discovery and regenerative medicine: Realizing the promise of three-dimensional organoids

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Three-dimensional organoids derived from human induced pluripotent stem cells (iPSCs) hold tremendous promise in transforming our understanding of human biology and disease, revolutionizing drug development, and ultimately, providing solutions for end-stage diseases through transplantation-based therapies. The ability of iPSC-derived organoids to replicate the architecture and physiology of human organs has established them as a valuable tool in the fields of developmental biology, personalized medicine, and drug discovery.

Despite their potential, current organoid culture methods face technical and conceptual limitations, including high variability and a reductionist approach that fails to account for complex interactions between parenchymal and non-parenchymal cells in native tissue microenvironments.

At the Center for Stem Cell and Organoid Medicine (CuSTOM) at Cincinnati Children's Hospital Medical Center (CCHMC), we have overcome these limitations by engineering next-generation organoids with cellular complexity, physiologic parameters, and higher-order functions similar to native tissues, as well as connections to other organs. Furthermore, we have implemented automation techniques and suspension cultures in shear-free bioreactors, which allow for efficient and scalable generation of large numbers of organoids at a low cost.

Our advancements bring us closer to realizing the full potential of organoids in drug discovery, personalized medicine, and regenerative medicine, including their use as tissue replacement therapies.

Presentation: Oral

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Lung-on-a-chip with an IOS-PU film to explore the effect of mechanical stretch on cell deformation and proliferation of alveolar epithelial cells

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After lung injury, the extracellular microenvironment plays a vital role in the self-healing process of lung tissue. Reliable *in vitro* lung microenvironmental models are of great importance for studying the basic biology of the lung as well as for therapeutic trials and drug testing. However, rare devices can fully reproduce the microenvironment, including mechanical stretch dynamically and cell-cell interface in the lung tissue until now [1,2]. Here, we present a biomimetic device with mechanical response and cell-cell interface to better simulate human pulmonary alveoli's three-dimensional architecture and function. This alveoli-simulating device comprises a porous, thin, and flexible film with an inverse opal structured polyurethane (IOS-PU) film which can give real-time observation of the degree of mechanical stretch. This device with mechanical response mimics the *in-vivo* interface between alveolar epithelial cells and vascular endothelial cells. With this device, we found the synergistic effect of mechanical stretch and vascular endothelial cells (ECs) on cell flattening and nucleus flattening in alveolar epithelial type 2 (AT2) cells, which are essential events during alveolar development. We also observed the synergistic effects of mechanical stretching and ECs on the proliferation of AT2 cells, which later influenced the repair process of lung injury [3]. Our research provided a reliable device for studying the mechanical perception during the repair process after *in-situ* injury in the lung and proposed new guidelines about drug targets in clinical therapy.

References

[1] Hogan, B. L., Barkauskas, C. E., Chapman, H. A. et al. (2014). Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell 15*, 123-138.

[2] Zhu, Y., Sun, L., Wang, Y. et al. (2022). A biomimetic human lung-on-a-chip with colorful display of microphysiological breath. *Adv Mater 34*, e2108972.

[3] López-Alonso, I., Blázquez-Prieto, J., Amado-Rodríguez, L. et al. (2018). Preventing loss of mechanosensation by the nuclear membranes of alveolar cells reduces lung injury in mice during mechanical ventilation. *Sci Trans Med 10*.

Presentation: Poster

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PERCEPT: A Parkinson's disease brain-chip model to unveil polyphenol metabolites potential

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Parkinson's disease (PD), the most debilitating motor degenerative disorder worldwide, still comprises a massive socioeconomic burden, holding a complex multifactorial etiology. Despite massive efforts, PD remains cureless, rendering critical the discovery of new treatments. Polyphenols have emerged as pleiotropic compounds with proven benefits against neurodegenerative diseases. Which polyphenols/bioavailable metabolites are responsible for the observed outcomes, and how, is still unknown.

We identified gut-derived polyphenol metabolites (PMs) as abundant in the bloodstream after the consumption of a polyphenol-rich meal [1]. Such PMs are transported across the blood-brain barrier (BBB), further metabolized [2], and present protective effects against neurodegeneration and neuroinflammation [2], particularly in a 3D neuronal model of PD [3]. However, PMs mechanisms in a more comprehensive and translatable model of the full brain were never explored before.

In PERCEPT, this knowledge gap will be filled using new microfluidic systems of a brain-on-a-chip, particularly tailored to recapitulate *in vivo* PMs pharmacokinetic profile and brain architecture. The new two-compartment model will employ a dopaminergic insult into human brain microvascular endothelial cells (HBMEC), and dopaminergic neurons (LUHMES) [3], with astrocytes (HASTR/ci35) and microglia (HMC3); parallelly, dopaminergic neurons differentiated from human iPSC which aggregate a-synuclein will also be used [4]. PMs (individually or as a mixture) will be infused from the blood site and their BBB transport assessed. PMs/PMs mixture role in tackling PD-related hallmarks will be uncovered, as BBB impairment, dopaminergic cell death, oxidative stress, neuroinflammation, cell crosstalk, a-synuclein aggregation.

PERCEPT will provide physiologically relevant insights for the nutritional management of PD: it foresees unveiling PMs' physiologic mode of action against PD. In the future, PERCEPT hold the potential to set the foundations for nutritional guidelines in preventing/ delaying PD appearance in patients, potentially revolutionizing the way we currently battle degenerative disorders.

Acknowledgments: to EU, for ERC – Grant No. 804229, through IMMUPARNET (CA21117); to FCT, for financial support of R.C. (PD/BD/135492/2018) and D.M. (2021.05505.BD), through PeX – 2022.02127.PTDC (PERCEPT) and through the R&D unit iNOVA4Health (LISBOA-01-0145-FEDER-007344; UIDB/ 04462/2020), and LS4FUTURE Associated Laboratory (LA/P/ 0087/2020).

References

[1] Pimpão (2015). *Br J Nutr.*
[2] Figueira (2017). *Sci Rep*.
[3] Carecho (2022). *Mol Nutr Food Res*.
[4] Zasso (2018). *Stem Cells Dev*.

Presentation: Poster

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An MPS device for *in vitro* peripheral neurotoxicity assessment based on morphological and electrophysiological characteristics

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Microphysiological system (MPS) is an *in vitro* culture technology that reproduces the physiological microenvironment and functionality of humans and is expected to be applied for evaluating drug efficiency/toxicity. In this study, a microfluidic culture device and related evaluation methods (i.e., deep learning for image analysis, and complementary metal oxide semiconductor microelectrode array (CMOS-MEA) technology) were developed to construct a rapid assessment platform for peripheral neuropathy caused by typical anticancer drugs.

COP (Cyclo olefin polymer), which has excellent observability and low drug adsorption, is used as the material of the present microfluidic device, and the device is manufactured by direct photobonding (R) method, which would induce no effect on the culture evaluation system due to the elution of the adhesive. Primary rodent dorsal root ganglia were cultured in the microfluidic device that separated the cell body and neurites, and morphological changes in the neuritis were analyzed using immunofluorescence imaging. Successful culture of separated neurites in the microfluidic device for more than 1 month indicated that this test process, including culture, drug stimulation, and fluorescence observation, results in a viable outcome.

Firstly, cultured neuron samples were treated with several representative anticancer drugs known to cause peripheral neurotoxicity (i.e., vincristine, oxaliplatin, and paclitaxel), and morphological changes in the neuritis were analyzed using AI deep learning for image analysis. After training, AI could identify morphological changes in the neurites caused by each compound and precisely predict toxicity, even at low concentrations. For the testing compounds, AI could also precisely detect neurotoxicity based on neurite images, even at low concentrations.

In addition, the conduction of electrical signal was measured in cultured neuron samples using a CMOS-MEA system with 236,800 electrodes, which provides high spatiotemporal resolution. After administration of anticancer drugs, the conduction pathway along each single neurite was successfully tracked and an acute change in conduction velocity was verified.

These results suggest that the present microfluidic culture system combined with deep learning and CMOS-MEA is a useful platform for *in vitro* neurotoxicity assessment.

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Microphysiological pancreas-on-chip platform with integrated sensors to model endocrine function and metabolism

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Pancreatic *in vitro* research is of major importance to advance mechanistic understanding and development of treatment options in the field of Diabetes Mellitus (DM) [1,2]. Here, we present a PMMA-based microphysiological system aiming to model the complex physiological microenvironment of pancreatic islets *in vitro* with concurrent real-time and *in situ* read-out possibilities.

The tailored microfluidic system enables self-guided trapping of single islets at defined locations to enable analysis on-chip. Pancreatic beta cells were assembled to 3D cell clusters, so called pseu-

do-islets, and injected into the tissue chambers using hydrostatic pressure-driven flow. They were further embedded within an ECM-like hydrogel emulating the physiological microenvironment and allowing for the integration of further tissue-relevant cell types around the pseudo-islets; thereby mimicking the native microenvironment towards a microphysiological pancreas-on-chip platform.

Non-invasive real-time monitoring of the oxygen environment on-chip was established utilizing integrated contactless optical sensors. To monitor insulin secretion kinetics in response to glucose stimulation in a time-resolved manner, an automated cycling of different glucose conditions was employed. High glucose treatment caused an increase in insulin secretion and oxygen consumption confirming cell functionality and higher metabolic activity of glucose-stimulated pseudo-islets on-chip.

As a proof-of-concept application the effects of two antidiabetic medications, a sulfonylurea, and a glucagon-like peptide 1 receptor agonist, were tested. In line with their mechanism of action the two drugs changed insulin secretion dynamics of the cells on the platform.

The presented MPS is a promising tool supporting 3-D tissue culture of pancreatic islet models in combination with further relevant tissue components, while providing the opportunity for non-invasive real-time and *in situ*-monitoring of the tissue on-chip.

References

[1] Zbinden, A. et al. (2020). Non-invasive marker-independent high content analysis of a microphysiological human pancreas-on-a-chip model. *Matrix Biol 85*, 205-220.

[2] Rogal, J. et al. (2019). Stem-cell based organ-on-a-chip models for diabetes research. *Adv Drug Deliv Rev 140*, 101-128.

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Modelling of human microphysiological skin system for preclinical evaluation of drug molecules

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Tissue engineering has enabled *in vitro* construction of human skin models (SM). However, existing models have limitations in emulating real human skin for preclinical assessments due to their functional simplicity. Thus, we have developed a single compartment microphysiological system, using a microfluidic device (Indian design patent no. 279195). Furthermore, we have also used static devices, having similar dimensions to develop SM. These devices were employed to compare the growth and differentiation of SM under the influence of nutrient circulation versus in static conditions. Morphological characterization of SM demonstrated spatial orientation of two distinct skin layers (i.e., epidermal and dermal) after 11 days of culture. Hematoxylin and eosin followed by immunofluorescence staining were performed to understand the detailed physiology and tissue-specific markers of SM in both devices. We also have performed biophysical validation of SM, which significantly increased under the influence of the flow operating conditions, as confirmed via the TEER values and rheological parameters. The performance of the developed SM was studied by evaluating the cumulative permeability (product of diffusivity and solubility) of drug molecules. To understand the diffusion kinetics of drug molecules through the developed skin layers in both devices, we developed closed form analytical model and validated it with experimental results, in terms of the outlet concentration of the model drug. A good agreement between the analytical and experimental results was observed as: ± 8.7%. Results suggested that the developed skin-on-chip model may be employed as an excellent alternative to the currently used animal skin models.

References

[1] Zoio, P. et al. (2022). Biomimetic full-thickness skin-on-a-chip based on a fibroblast-derived matrix. *Micro*. doi:10.3390/micro2010013

[2] Risueño, L. et al. (2021). Skin-on-a-chip models: General overview and future perspectives. *APL Bioeng*. doi:10.1063/5.0046376

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Engineering glycosaminoglycan-based hydrogels to modulate microvascular network formation *in vitro*

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Introduction: Vascularization is a crucial factor in the growth and maintenance of tissue-engineered models. A common method for generating perfusable microvascular networks *in vitro* is through the self-organization of endothelial cells. However, this approach

has so far been successful only using biopolymer-derived matrices, which have lot-to-lot variability, complex signaling cues, limited stability, and poor tunability. In this study, we applied a modular biohybrid hydrogel based on glycosaminoglycans (GAGs) and 4-arm poly(ethylene glycol) (starPEG)-peptide conjugates to understand the impact of synthetic matrix properties and heterotypic cell-cell interactions on the formation, functionality, and stability of 3D microvascular networks *in vitro*.

Methods: Human vascular endothelial cells (HUVECs) expressing GFP, alone or in combination with stromal cells, were encapsulated in soft hydrogels cleavable by matrix-metalloproteases (MMPs) containing an adhesive peptide (CWGGRGDSP, or cRGD), and varying concentrations of pro-angiogenic growth factors: VEGF165, bFGF, and SDF1α [1,2]. The cells were cultured and imaged for up to 28 days.

Results: Our results showed that glycosaminoglycan sulfation and concentration were major determinants influencing the capillary morphogenesis of HUVECs in soft hydrogels. Mathematical modeling revealed that these parameters modulated the endothelial network formation by controlling free VEGF availability. We further found that functionalizing the hydrogels with triple heparin-binding pro-angiogenic growth factors and co-culturing HUVECs with bone marrow-derived mesenchymal stromal cells or human lung fibroblasts increased the density, total vessel length, and branching points of microvasculatures. Co-culture with stromal cells was particularly critical in reducing vascular network regression, regardless of stromal cell identity. Furthermore, by fine-tuning the stiffness of hydrogels, we were able to develop microvascular networks with persistent and perfusable lumens.

Conclusions: In conclusion, we have applied a tunable star-PEG-heparin hydrogel platform to identify synthetic matrix properties and heterocellular communication that govern vascular morphogenesis as well as microvascular network stability and perfusibility. The obtained results can pave the way for advanced vascularized tissue and organoid models based on thoroughly tunable biomaterials.

References

[1] Chwalek, K., Tsurkan, M. V., Freudenberg, U. et al. (2014). *Sci Rep 4*, 4414.

[2] Limasale, Y. D. P., Atallah, P., Werner, C. et al. (2020). *Adv Funct Mater 30*, 202000068.

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Nanoparticles stokes radius assessment through permeability coefficient determination within a new stratified epithelium on chip model

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Tissue barrier permeability is essential in the selective transport across the epithelium, affecting the absorption of a drug into the bloodstream, and represents a useful tool to quantify the amount of drug, cosmetic substance, or chemical that may cross the skin or the systemic circulation of the human body for pharmaceutical or cosmetic purposes, or for toxicological studies. On this regard, microfluidic devices and organ-on-chip technologies are becoming more important to generate reliable data. We have selected an already fabricated COP microdevice to grow an epidermal layer formed by the immortalized keratinocyte cell line HaCaT to develop an epidermal on chip model that allows easy permeation quantification of multiple pharmaceutical dosages from different fluorescent-labelled active molecules at the same time. We have validated the accuracy of the model by correlating the stoke ratio of several compounds with different molecular weights with their empirically determined permeability coefficient. Moreover, we have determined the stoke ratio from several synthesized fluorescent labelled nanoparticles by empirically quantifying their permeability coefficient, confirming the calculated stokes ratios by electron microscopy. The further development of the presented epithelia-on-chip platform will enable the cost-effective and reliable determination of Stokes radius in a more physiological microenvironment from topical ingredients for pharma and cosmetic industry.

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Culturing topologically controlled neuron and neuron-astrocyte networks on microelectrode arrays

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Neurons and astrocytes are traditionally cultured as dissociated cultures *in vitro*. The neuronal network growth is "random" in these cultures, and therefore, the propagation of information is unknown. Microelectrode arrays (MEAs) can detect extracellular action potentials generated by neurons. However, the spatial resolution of the signals is limited in the randomly grown cultures. We cultured rat primary neuron and neuron-astrocyte co-cultures [1] on MEAs with a highly topologically controlled system, where a thin microfabricated polydimethylsiloxane (PDMS) microchannel structure allows neurons and astrocytes to grow only at certain locations and to certain directions [2,3]. The PDMS sheet consisting of 15 independent microcircuits was placed on the top of a 60-electrode MEA, which allowed studying topologically known neuronal networks. We plated various ratios of astrocytes to these microcircuits and evaluated the effects of astrocytes on neurons. The cultures contained 50% neurons and astrocytes, 80% neurons and 20% astrocytes, or only neurons without separately added astrocytes. These cultures were grown and followed up by MEA measurements for 29 days. The cultures were also assessed using imaging methods, such as immunocytochemical staining and scanning electron microscopy to evaluate cell growth in the microcircuits. We observed that astrocytes enhanced neuronal viability, growth, and the electrophysiological activity, evaluated with spike rates and burst rates. Furthermore, astrocytes modulated the connectivity of the networks. In conclusion, "bottom-up", well-defined neuronal microphysiological systems are a promising technique to study neuronal information propagation and the effects of astrocytes on neurons and circuits. Moreover, such microcircuit systems can be used for studying and validating network connectivity methods as the underlying neuronal network structure is known.

References

[1] Ahtiainen, A. et al. (2021). Astrocytes exhibit a protective role in neuronal firing patterns under chemically induced seizures in neuron-astrocyte co-cultures. *Int J Mol Sci 22*, 12770. doi:10.3390/ijms222312770

[2] Forró, C. et al. (2018). Modular microstructure design to build neuronal networks of defined functional connectivity. *Biosens Bioelectron 122*, 75-87. doi:10.1016/j.bios.2018.08.075

[3] Girardin, S. et al. (2022). Topologically controlled circuits of human iPSC-derived neurons for electrophysiology recordings. *Lab Chip 22*, 1386-1403. doi:10.1039/D1LC01110C

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In vitro oral and topic absorption toxicity test standardization using 3D cell cultures and microfluidic systems

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Organ-on Chip is a result of tissue engineer and microfluidic convergency, acting as an effective solution to pursue new methodologies for drug discovery and personalized disease treatments. The high cost of drug development demands the need to develop more predictive tissue models using human cells to determine drug efficacy and safety in advance of clinical testing. However, a more predictive model requires the integration of different tissues, dynamic cell environments and cellular communication to the expression of high-fidelity organ function. In this context, the microfluidic technology is poised to fill the gaps in drug screening by offering predictive human tissue models with methods of sophisticated tissue assembly. Here we propose a junction of three different 3D tissue engineered cultures (skin, intestine, and liver) in a 3-organ-on chip microfluidic device to propose a methodology to verify topic or oral drug administration and liver toxicity. For this, we developed models of human reconstituted skin, intestinal barrier, and liver spheroids, which were deep characterized in terms of histology, morphology and functionality. Our results show that our models are functional and mimetites functions of the real organs. The Chip integration of all the tissues on the chip was well succeeded and improved viability of the 3D cultures. After treatment with known toxic substances and, we observed absorption of drugs, which caused liver injury, as expected. In conclusion, here we present a new methodology to screen liver toxicity before animal testes in two contexts, oral and topic administration of compounds.

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Use of a 3D-*in vitro* model for the assessment of liver metabolism related to neurotoxicity of occupationally relevant chemicals

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Several neurological disorders have been linked to occupational exposure to chemicals. Propylene glycol ethers are commonly used as mixtures of a non-toxic α-isomer and a β-isomer that is oxidized to a potential noxious acid metabolite via the alcohol dehydrogenase (ADH) and the aldehyde dehydrogenase (ALDH). However, studies about the neurotoxicity of these solvents are rare. Knowing the rate of solvent metabolism is important to estimate the metabolite exposure for the brain. Here, we studied the toxicity of selected solvents and their corresponding acid metabolites on liver cells and proved the 3D *in vitro* HepaRG liver model to predict hepatic metabolism.

The synthesis and release of albumin as a functional hepatocyte marker were detected by immunostaining and ELISA albumin assay. The activities of metabolic phase I enzymes were confirmed with known substrates. Presence of the ADH and ALDH in HepaRG was determined on gene level, protein level, and activity confirmed in metabolism studies. Michaelis-Menten-Kinetic of metabolite formation was studied with liver S9 fractions. After exposing the 3D HepaRG model to the solvents, we measured metabolite concentration by LC-MS.

Cytotoxic studies showed decreased cell viability accompanied by decreased cellular albumin levels, whereby the 3D cultures were more sensitive than the 2D cultures. The 3D HepaRG cells were able to perform phase 1 metabolism and metabolism via ADH and ALDH. Moreover, liver S9 incubations served as system to estimate the enzyme kinetic of metabolite formation. Finally, formed metabolites were measured in the 3D HepaRG model and kinetic parameters calculated.

In conclusion, we established a metabolically competent 3D HepaRG model suitable to measure metabolite formation. Future steps include the integration of our *in vitro* toxicity data into a physiological based toxicokinetic (PBTK) model to predict human systemic and brain exposures and thus support risk assessment of occupationally relevant chemicals.

References

Brown, R. C., Lockwood, A. H. and Sonawane, B. (2005). Neurodegenerative diseases: an overview of environmental risk factors. *Environ Health Perspect 113*, 1550-1556.

ECETOC (2005). The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition). Volume II – Substance Profiles. Tech Rep II (95), 159-162.

Benet, L. Z., Zia-Amirhosseini, P. (1995). Basic principles of pharmacokinetics. *Toxicol Pathol 23*, 115-123. doi:10.1177/019262339502300203

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Antigen specific antibody responses in a human lymph node-on-a-chip for drug development research

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Microfluidic technology is a crucial toolbox for creating physiologically relevant cues to traditional cell culture, including long-term gradient stability and continuous perfusion. Furthermore, it allows patterning of cell layers as stratified co-cultures devoid of artificial membranes to capture the complex tissue structures and organization observed *in vivo*. Currently, there are no translatable *in vitro* models to assess human T cell-Dependent Antibody Responses (TDAR). Previous *in vitro* systems have failed to recapitulate *in vivo* B cell responses and to provide a proper assay window to study induction of immune responses upon primary antigen exposure. Therefore, the MIMETAS OrganoPlate platform was used to culture human immune and supporting cells, in order to recapitulate *in vivo* TDAR responses in an *in vitro* human lymph node. We established a human lymph node showing changes in immune cell development and humoral immune responses upon antigen exposure. B cell and T cell compartments could be observed, including reorganization upon antigen exposure and robust immune responses. Development of follicular immune cell subsets, germinal center formation, plasma cell differentiation and memory cells were observed. OrganoPlate-grown human lymph nodes are suitable for developing complex systems to mimic *in vivo* immunological responses to antigens or immunomodulatory therapeutics. This system is suitable for screening using real-time imaging, flow cytometry, ELISA and other quantification methods. Our microfluidic technology serves as a powerful platform for studying physiological disease mechanisms in human lymph nodes and therapy development.

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3D human retinal organoid model for the study of early diabetic retinopathy

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Diabetic retinopathy (DR) is a major complication of diabetes and a leading cause of visual impairment in the working-age population. This is a degenerative disorder where hyperglycemia leads to morphological and functional impairment of specific retinal cells. The available treatments only target the later stages of the disease involving vascular defects such as macular edema or neovascularization. However, retinal neurodegeneration and inflammation are now known to precede vascular alterations. Therefore, it is urgent to identify new therapeutic approaches acting on early stages and halting disease progression.

In the last decade, the development of reliable cell culture models which resemble the complexity of the retinal tissue has significantly improved. Namely, three-dimensional (3D) retinal organoids derived from human induced-pluripotent cells (hiPSCs) recapitulate the cellular organization and complexity of the human retina.

Here, we used human retinal organoids to generate a model of early DR. In this model, we observe the loss of ganglion and amacrine cells by exposing cells to high glucose levels. Glial reactivity and inflammation were also reproduced, with increased expression of the VEGF and IL-1β encoding genes, and enhanced MCP-1 secretion. Moreover, there are increased levels of reactive oxygen species (ROS) accompanied by the activation of key enzymes involved in antioxidative stress response. These changes are all well-established molecular and cellular features of early DR. Its reproducibility in our model opens new perspectives to its use for studying the mechanisms and for developing targeted therapeutic strategies against neurodegeneration and inflammation in early DR and delaying the progression of the disease.

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Development and validation of an airway-infection-on-a-chip microfluidic platform

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Antibiotic resistance has been declared a severe public health problem by the World Health Organization (WHO). This problem is particularly pressing in the treatment of chronic bacterial respiratory infections, such as those caused by *Haemophilus influenzae*, due to increasing ampicillin resistance. In fact, *H. influenzae* biofilm growth favors chronicity of the infection, antibiotic resistance, and therapeutic failure [1,2]. Nowadays, effective strategies to study biofilm formation in the human lower airways remain limited. 2D cell cultures allow investigating host-pathogen interactions but lack physiological and anatomical relevance. Animal models of pulmonary infection can be a good approach, but remain costly, difficult to monitor, and face increasing ethical restrictions. Therefore, it becomes necessary to develop new platforms for studying chronic bacterial infections. During the last decades, organ-on-a-chip technologies have emerged as promising human models to simulate the interactions occurring in the body, improving the existing, conventional methods. In this work, we introduce an airway-infection-on-a-chip platform, a microfluidic device inspired by the existing airway-on-a-chip devices which, notably, allows modelling chronic bacterial infections by the inclusion of biofilm-forming reservoirs. Such reservoirs are connected to the alveolar device by a rotatory valve, which allows independent, but simultaneous culturing of bacteria and cells in the same chip and, when desired, connects both compartments to elicit interactions between both biological entities. The design and optical properties of these devices allow studying cell-bacteria interactions in a chronic-like setting in an easy and more compact way, which could help in the testing of new antibiofilm treatments. We present the conceptual design and provide practical guidelines for the fabrication and functionalization of these devices. Then, we illustrate the use of this device to study host-pathogen interactions within the human lower airways, by using *H. influenzae* biofilms as a proof-of-principle.

References

[1] Bowler, P.G. (2018). *J Wound Care 27*.
[2] Marcinkiewicz, J. et al. (2013). *Pol Arch Med Wewn*.

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The application of ImmuLUNG™ in inhalation safety assessments and organ-on-chip platforms

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Human airway epithelial cell culture models have been the basis of *in-vitro* inhalation toxicity testing methods for the past twenty years. Whilst these can be beneficial for understanding how inhaled substances interact with the airways, it's becoming increasingly evident that tissue-resident immune cell (alveolar macrophage) responses are important for recognising the full safety implications of substances in the alveolar region of the lungs. Many 3D alveolar model airway models either lack immune cells, include a non-lung specific leukocyte component, or have a sub-optimal barrier for the alveolar epithelial component [1]. It's well established that a dynamic culture environment can create more physiologically representative tissue models [2]. ImmuLUNG™ is a human 3D epithelial-macrophage *in-vitro* cell culture model of the alveolus. This study aimed to characterise ImmuLUNG™ under static and dynamic culture conditions to assess its suitability for incorporation into a micro-physiological platform.

ImmuLUNG™ was constructed on 0.4 µm membranes and cultured in static (Transwell® inserts) and dynamic conditions (AKITA® Plate, Finnadvance). ImmuLUNG™ was characterised for morphology, viability, and functionality of immune and epithelial cells in static and dynamic culture conditions to ascertain the physiological relevance of both platforms to the human alveolus. Viability of the systems was evaluated for up to 7 days, with cytotoxicity below 27% in both conditions. Epithelial barrier integrity was assessed and showed TEER > 1000 $\Omega.cm^{-2}$ (static and dynamic), while Papp was reported 0.4 x 10^{-6} cm^{-1} and 0.18 x 10^{-6} cm^{-1} for static and dynamic conditions, respectively, using fluorescein as a tracer. Macrophages incorporated into the model maintained the non-proliferative character and macrophage morphology and were adherent in dynamic conditions for up to 3 days.

In conclusion, ImmuLUNG™ provides a physiologically relevant *in-vitro* representation of the human alveolus with a functional epithelial barrier and tissue-resident macrophage features. This study has demonstrated that ImmuLUNG™ retains key features in static and dynamic culture environments, making it useful within the standard determination of airway toxicity (barrier integrity) alongside immune responses to support the determination of the mechanism of substance toxicity and tissue pathology.

References

[1] Barreiro Carpio, M. et al. (2021). https://www.frontiersin.org/articles/10.3389/fbioe.2021.773511/full

[2] Shrestha, J. et al. (2020). doi:10.1080/07388551.2019.1710458

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A multi-compartment lung-on-chip model to study the (patho) physiological relevance of biological hydrogels

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Biological hydrogels such as mucus exert critical protective and homeostatic functions in the human lung epithelium. Abnormal alterations of its composition and secretion are linked to increased susceptibility to acute and chronic lung diseases. As of today, human disease models that allow studying of the relevant interdependencies are largely lacking resulting in knowledge gaps as to how biological hydrogels contribute to certain disease states [1]. To close this gap, we developed a dynamic multi-compartment lung model that enables the co-cultivation of human alveolar and bronchial epithelial tissue and facilitates physiological hydrogel expressions. The connection of this model to a microfluidic chip platform further allows the application of breathing mechanics resulting in a setup of high biomimicry. One of the compartments is a well-characterized bronchial epithelial model composed of normal human lung fibroblasts (NHLFb) and normal human bronchial epithelial cells (NHBE) [2]. This segment is subsequently surrounded by a monolayer of human primary alveolar epithelial cells (HPAEpic) emulating the bronchial-alveolar transition zone. This multi-compartment chip setup is highly versatile and can be adjusted to different culture conditions such as air-liquid-interface (ALI). We could show that after shifting the alveolar compartment to ALI a significant increase of transepithelial electrical resistance (TEER) over 1400 $\Omega*cm^2$ together with the uniform expression of essential barrier integrity markers ZO-1, e-cadherin, and actin occurred. Examination of this network helps to explore the effect of different mechanical stimuli provided by the microfluidic system. Additional immunofluorescence staining verified an abundant population of alveolar type II cells, which are responsible for the alveolar hydrogel (surfactant) production. We demonstrated the expression of surfactant protein B and prosurfactant protein C determined by RNA-Seq analysis and IF staining. Moreover, preliminary proteomic analysis revealed high similarities between the secreted mucus of the bronchial epithelial model and native lung mucus. Currently, we are further optimizing this culture by leveraging flexible colla-

gen membranes to facilitate physiological deflection as well as the versatility of stem cell-derived alveolar epithelial cells to further enhance the predictivity of our setup.

References

[1] Budden, K. F. et al. (2017). *Nat Rev Microbiol 15*, 55-63.
[2] Charbaji et al. (2021). *Small 17*. e2007963.

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Microphysiological systems in biomedical research

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According to the last report on the use of animals for scientific purposes in EU Member States, about 70% of animals were used in basic, applied and translational research in 2019 in the fields of human and veterinary medicine.

Recent scientific developments in the biomedical research field have resulted in a new generation of advanced models that better address the specific features of human diseases. The emerging trend is to exploit the human relevance of advanced non-animal models to provide strong mechanistic rationale for diagnostic, preventative and therapeutic interventions, thus representing an important step towards precision medicine tailored to the patient.

To promote the use of emerging non-animal models in biomedical research, EURL ECVAM carried out a series of systematic reviews on seven disease areas, including *in vitro* methods based on human cells and engineered tissues and *in silico* approaches employing computer modelling and simulation.

The results have been published [1] and seven curated databases, capturing a total of 3050 non-animal models, are now available to the scientific community for the following disease areas:
– respiratory tract diseases
– breast cancer
– neurodegenerative disorders
– immuno-oncology
– immunogenicity testing for advanced medicinal therapy products
– cardiovascular diseases
– autoimmune diseases

Among these, more than 1000 *in vitro* models can be classified as microphysiological systems (MPS), including 3D cell (co-)cultures, organoids, organ-on-chip models, multi-organ models, as well as related biotechnologies, such as bioreactors. The fast-evolving field of breast cancer has been identified to be the most model-rich, including 823 *in vitro* models, the majority of which (55%) are MPS.

The development of MPS can bring new tools in pharmacology and medicine that increase the efficiency and safety of testing and development. Characteristic is the example of organ-on-chip that can facilitate the pharmacokinetics and allow for efficient the drug screening processes.

While this analysis illustrates the significant contribution of the MPS to the biomedical field, the translation from academic research to widespread use is still ongoing and evolving. There is a need to improve standardisation and transferability of the methods, as well as to use of best practices for the reporting of methods.

Reference

[1] Biomedical Research – ECVAM page: https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/biomedical-research_en

Presentation: Poster

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SARS-CoV-2 variant infection differences between static and microphysiological system models of the human lung

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The coronavirus disease 2019 (Covid-19) pandemic had an immense impact on human health and the economy [1] and presented an immediate need to understand the biology of SARS-CoV-2 virus and identify effective therapeutics against the disease. Despite a successful vaccine roll-out, the threat of SARS-CoV-2 to human health is still present and better models to study the disease are needed. Traditional *in vitro* immortalised cell culture models are unable to effectively replicate complex tissues and human physiological environments [2] and alternatives such as animal models, are expensive and have fundamental differences to the human host which prevent direct comparisons to be made. This is particularly evident for SARS-CoV-2, as viral adaptations may have allowed more effective infection in humans compared to rodents [3]. Dynamic microphysiological systems (MPS) have the potential to bridge the knowledge gap between traditional *in vitro* models and human clinical trials to enable us to understand SARS-CoV-2 infection and identify effective therapeutics.

We have therefore undertaken experiments to compare a "dynamic" lung-on-a-chip system to traditional "static" *in vitro* cultures. Co-cultures of human primary epithelial and endothelial cells were differentiated at air-liquid-interface (ALI) for 14 days

either in static conditions or using the CN Bio PhysioMimix™ system, before being infected with pre-alpha, alpha or delta SARS-CoV-2 variants. We will present data which shows significant differences between viral recovery and replication rates in static vs dynamic lung models. We also compared the infection of bronchial and alveolar MPS with SARS-CoV-2 variants and demonstrated infectivity differences between the distinct respiratory areas through analysis of live viral recovery rates, qPCR and gene expression data. These data will be discussed within the clinical context of the pathology and transmission of SARS-CoV-2.

References

[1] Miyah, Y., Benjelloun, M., Lairini, S. et al. (2022). COVID-19 impact on public health, environment, human psychology, global socioeconomy, and education. *Scientific World Journal 2022*, e5578284.
[2] Kaur, G. and Dufour, J. M. (2012). Cell lines. Valuable tools or useless artifacts. *Spermatogenesis 2*, 1-5.
[3] Boechat, J. L., Chora, I., Morais, A. et al. (2021). The immune response to SARS-CoV-2 and COVID-19 immunopathology – Current perspectives. *Pulmonology 27*, 423-437.

Presentation: Poster

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Tomographic volumetric bioprinting of 3D pancreatic cancer models

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The processing of biomaterials by additive manufacturing techniques is investigated to obtain 3D bioengineered *in vitro* tissue models which truly recapitulate the biological and biophysical complexity of human microenvironments [1]. Hydrogels are considered excellent biomimetic platforms, mainly due to their extracellular matrix-like features as viscoelasticity, high water content, tunable mechanical properties and cell adhesive motifs [2]. Gelatin-methacryloyl (GelMA), a photo-crosslinkable form of gelatin, has emerged as common bioink due to its rapid crosslinking rate and excellent thermostability [3]. Here, GelMA is processed by volumetric tomographic bioprinting, an emerging light-based technology, to fast-fabricate cellularized 3D constructs with high-resolution and complex geometry. Particularly, we adopted this innovative technique to develop a biomimetic 3D *in vitro* model of the human pancreatic gland for the study of pancreatic cancer.

Human fibroblasts (HFF1) were embedded in a GelMA hydrogel, prepared tailoring the polymer and the photoinitiator concentrations, and the complex glandular structure was obtained by volumetric additive manufacturing. Human pancreatic ductal epithelial cells (HPDE) stably expressing activated KRAS (HPDE-KRAS), were then seeded within the cavity of the bioprinted constructs. The stromal and pancreatic cancer cells crosstalk was evaluated by quantifying the expression of fibroblasts activation markers (i.e. alpha smooth muscle actin, α-SMA).

Results showed that fibroblasts remain viable up to 14 days within the GelMA constructs, while the HPDE-KRAS cells are able to entirely cover the inner walls of the structure. Finally, HFF1 embedded in GelMA hydrogel show an increment in α-SMA expression when co-cultured with HPDE-KRAS compared to HFF1 alone and to fibroblasts seeded with normal HPDE cells.

This fully human 3D *in vitro* model allows to recapitulate the complex microanatomy of the exocrine pancreas, for the study of pancreatic cancer.

References

[1] Kapałczyńska M et al. (2018). *Arch Med Sci 14*, 910-919.
[2] Monteiro, M. V. et al. (2022). *Biomaterials 287*, 121653.
[3] Yue, K. et al. (2015). *Biomaterials 73*, 254-271.

This work was partially supported by the European's Union Horizon 2020 research and innovation program (grant #814495). This manuscript reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.

Presentation: Oral

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Gelatin methacryloyl hydrogels: A versatile platform to recreate the 3D microenvironment of native tissues *in vitro*

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Collagen is the main component of the extracellular matrix of many tissues. It is widely investigated in tissue engineering due to its clear capability to recapitulate the native tissue/organ microenvironment. However, collagen suffers from high costs due to its difficult isolation and the non-aggressive conditions required to

avoid denaturation. Conversely, gelatin is a cheap collagen derivative. The collagen amino acid sequence is kept in gelatin, while its right-handed supercoiled structure is lost. Consequently, gelatin requires cross-linking to improve mechanical performances and stability in physiological conditions. In this contribution, we bulk-functionalized gelatin with methacryloyl moieties, obtaining gelatin methacryloyl (GelMA), which aqueous solutions (added with photoinitiator) undergo cross-linking through photo-irradiation (30-90 s, 365 nm, 10 mW/cm^2). To modulate gel stability and mechanical properties, we synthesized GelMAs with methacryloylation degrees within 55-100%, and we tuned hydrogel concentration within 5-10% w/V. To design *in vitro* bone tissue models, bioactive bio-inks were designed by adding nano-hydroxyapatite to GelMA solutions, mimicking bone inorganic and organic phases. Gels were loaded with a co-culture of murine osteoclasts and osteoblasts and microfabricated into multi-layered structures with adequate geometry to host prosthetic prototypes. Conversely, human-induced pluripotent stem cell-derived cardiomyocytes and human coronary artery endothelial cells were co-cultured into GelMA gels to develop *in vitro* cardiac tissue models for cardiotoxicity evaluation. 3D models were compared to traditional 2D co-culture and validated using doxorubicin. In the 3D environment, cells showed improved resistance (increased mitochondrial function, reduced ROS and LDH release) to doxorubicin toxicity compared to 2D co-culture, in accordance with literature [1]. Formulations validated in this work open the way towards the design of increasingly complex models of the bone-implant interface and ageing cardiac tissue models for cardiotoxicity assessment in the elderly population. Upon integration in sensorized bioreactors, these devices will become powerful tools to investigate therapeutic approaches, drug efficacy and toxicity.

This work was supported by the European's Union Horizon 2020 research and innovation program (grant #101037090, 814495). This manuscript reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.

Reference

[1] Edmondson, R. et al. (2014).

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Development and characterization of patient derived organoids from human breast tissue

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Breast cancer (BC) is the most frequently diagnosed cancer among women worldwide. Currently, 2D-cultured cell lines and patient-derived xenografts (PDX) are used to study BC tumor biology and drug discovery. As 2D cell lines cannot represent the 3D-complexity of human tissue, and PDX models are cost-prohibitive and time-consuming, there is need for better tissue mimetic models.

Organoids are 3D-cultured units that structurally/ functionally mimic corresponding human tissue [1]. Patient tissue-derived organoids (PDO) resemble source tissue in molecular features and may recapitulate patient-specific responses to therapies [2]. We report developing novel breast PDO lines from vendor-sourced fresh (≤ 24 h post-surgery) and cryopreserved (≤ 7 days post-surgery) tissue.

For each consented patient, tumor tissue as well as normal, tumor-adjacent (NTA) tissue was obtained. PDO were generated following a published protocol [3, with modification]. Briefly, samples were minced, digested with collagenase and filtered. Filtered tissue was resuspended in Matrigel (Corning) and cultured as small drops on plates in media specific for tumor or NTA [3, with modification]. PDO were passaged every 10-14 days and monitored using brightfield microscopy (Olympus) and/ or live cell imaging (Perkin Elmer). PDO were cryopreserved using Cryostor10 (StemCell) or breast PDO media (+DMSO) and thawed following in-house SOP to confirm viability. PDO were cleared for microbial/ viral contamination and analyzed for expression/ distribution of important biomarkers using confocal microscopy (Molecular Devices). PDO lines displayed key biomarkers like hormone receptors (ER, PR), cell surface (EpCAM, CD49f), cytoskeletal (cytokeratin 14, 19, E-cadherin, vimentin) and cell proliferation (Ki67). Transcriptome profiles were analyzed using vendor services (Azenta) and PDO were tested for susceptibility to anti-BC drugs, e.g., tamoxifen.

We have successfully generated the following PDO lines: (i) NTA ER+/PR+/HER2- (fresh tissue); (ii) NTA ER+/PR+/HER2- (cryopreserved tissue, 2 sets); (iii) tumor ER-/PR-/HER2- (cryopreserved tissue) and (iv) tumor ER+/PR+/HER2- (cryopreserved tissue). Our breast PDO lines would be highly useful in understanding tumor biology, BC progression, signal transduction, and drug discovery/ testing. Future directions would include scaling up of PDO processes and developing BC-specific assays.

References

[1] Zhao et al. (2022). *STAR Protocols.*
[2] Bleijs, M. et al. (2019). *EMBO J.*
[3] Dekkers et al. (2021). *Nat Protoc.*

Presentation: Poster

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Biofabrication of a glomerular 3D model by mimicking its functional core components

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Kidney failure affects a large population and is often associated with glomerular diseases. Glomeruli are the functional units of the kidney responsible for selective blood ultrafiltration through the glomerular filtration barrier. The current gold standard for *in vitro* glomerular models remains limited to recapitulate their structural complexity. Therefore, there is a great demand to develop a glomerular *in vitro* model with all relevant cell types and functional components. This project aims to fabricate an *ex vivo* 3D model of the glomerulus by recapitulating its functional core components by using bottom-up and top-down biofabrication techniques. The model will be utilized to investigate cell-cell communication and renal (patho-)mechanisms.

The project is split into four different work packages. First, a Poly-L-Lactic Acid (PLLA) electrospun nanofibrous membrane offered support to a co-culture of monolayered podocytes and glomerular endothelial cells (GEC) to recapitulate the glomerular filtration barrier. Different topographies (random and aligned) of PLLA-fibers and coatings were investigated regarding their effects on cell attachment spreading, and proliferation. Filtration functionality and cell-cell communication was tested in bioreactors under different flow conditions.

The second 3D model is based on spheroids. We demonstrated that spheroids consisted of different co-cultured glomerular cell types survive longer than mono-culture spheroids and arrange specifically within the spheroid. Moreover, 3D structure as well as co-cultivation enhanced extracellular matrix (ECM) production resulting in an ECM network formation. On the other side, common limitation of spheroids is the hypoxia of the inner mass and subsequent necrosis. To avoid insufficient supply of deep-lying cells, we integrated nanofibers in spheroids and thereby improved cell survival and proliferation.

In order to provide vascularization, a third model was established. Hollow capillary-like structures within GelMA created by the dissolving of polyoxazolines Melt-Electro-Writing-printed loops were seeded with GEC and podocytes. The creation of a liquid flow supported the differentiation of GEC and formation of cell-produced basement membrane.

Finally, all the components will be bioassembled in a complex 3D model to study different glomerular functions in parallel.

Presentation: Poster

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A cell tetraculture model combined with an air-liquid interface exposure system, as easy-to-use alternative technology for *in vitro* hazard assessment of respiratory sensitizers

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A tetraculture system representative of the human alveolar region has been set up combining alveolar epithelial cells (A549 cells) and macrophage-like cells (seeded on the apical side of a Transwell™ insert), with EA.hy926 endothelial-like cells (attached on the basolateral side of the membrane) and dendritic-like cells (DCs THP-1), floating underneath (Patent WO2018/122219 A1).

This orientation allows growing the apical side at the air-liquid interface (ALI) while maintaining the basolateral side in submerged conditions. The presence of DCs, as well as the cross-talking with the micro-environment and the presence of macrophage-like cells, make the system a promising candidate as alternative *in vitro* model for the assessment of respiratory irritants and sensitizers. Preliminary data after exposure to selected chemical compounds has shown that the exposure to respiratory sensitizers induces dendritic cells activation through expression of specific surface markers like CD54, CD86 and TSLPr, while the irritants do not. The model is currently being tested for its potential application also in the frame of safety assessment of nano and micro particles. In order to facilitate interlaboratory comparison thus contributing to the validation process of the model, a transportation medium has been developed. Briefly, different formulations have been tested to synthetise an alternative gelling agent based on animal-free raw materials suitable for long distance shipping of the cell co-culture. The final formulation (Patent WO20221/36383 A1) has been already used to send the coculture model within Europe (e. g Switzerland, Netherlands) with promising outcomes.

In addition, to reduce the amount of testing compounds used during the exposure phase, an improved exposure system based on an existing technology has been developed (Patent LU501054).

The new device allows a non-intrusive heating and temperature monitoring within the housing and quick temperature rising in the exposure chamber thus increasing the deposition rates and reducing the condensation of the aerosol on the casing walls. Moreover, a scaffold-like housing allows the placement of different formats of multi-well plates while a disposable pierced lid reduces the risk of chemical carryover and biological contamination.

Presentation: Poster

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Quantification of insulin response in a modular multi-organ chip approach: White adipose tissue-liver axis

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The molecular mechanisms of inter-tissue communication considering glucose and lipid metabolism as well as inflammatory cues are essential for understanding insulin resistance triggering events. Multi-organ-chips (MOCs) allow addressing organ-specific roles on the response to insulin and fed states while circumventing the complexity and non-human nature of animal models, and the limited sampling possibilities and of clinical studies. The aim of this work to develop and use a PDMS-free microphysiological modular OoC platform and, thereby, provide a human physiologically relevant *in vitro* insulin signalling model. Human subcutaneous white adipocytes and human microvascular endothelial cells were isolated from skin biopsies for the white adipose tissue (WAT)-chip while the HepaRG® cell line was used for the Liver-chip. We assessed insulin sensitivity in WAT-chips by overnight fasting followed by 30 minutes of perfusion with fed-state medium (insulin concentrations: 60-6000 nM) to identify the response threshold. 3D imaging of nuclei, cytoplasm, FOXO1 and GLUT4 allowed the quantification of the subcellular location of these markers upon insulin stimulation. Lactate and glycerol were measured from the chip effluents and the adipokine profile was performed using a multi-plex bead-based commercial kit. WAT- and Liver-chips were separately co-cultured with endothelial cells. The two-organ connection was characterized by immunofluorescence after 5 days of connection and perfusion of an optimized common media. The secretome of WAT-chips with and without endothelial cells was characterized for donor specific lipolysis, adipokine profile and cellular metabolic rate. Time-resolved insulin response using healthy post-prandial (fed) glucose (11 mM) and insulin (60-6000 nM) levels showed lipolysis inhibition, increase in lactate production, significant FOXO1 translocation from nuclei to cytoplasm, and translocation of GLUT4 to the cell membrane. The medium for the vascularized WAT-liver connection was optimized to maintain adipocyte morphology and unilocularity, endothelial expression of CD31 and to allow for a continuous monolayer covering both tissues under fluidic flow. HepaRG cells were Albumin, MRP-2 and E-cadherin positive in the co-culture medium and when connected downstream of the WAT-chip. Applying tailored MOC to the study of insulin resistance in a tunable tissue and molecular perspective will improve the mechanistic understanding of metabolism in a personalized and/or population level.

Presentation: Oral

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Modeling clinically relevant neural tube defect risk using RosetteArray™ technology

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Neural tube defects (NTDs) remain the second most common congenital malformation. Given their complicated multifactorial etiology and species differences in neural tube closure, current *in vitro* models (mainly gene altered rodents) have struggled to investigate clinical NTD pathophysiology and interventions due to their limited scale and non-human background. Most rodent models depict failure in forebrain neural tube closure (anencephaly), while spinal defects dominate human presentation (spina bifida). Furthermore, no single gene has been identified that causes isolated NTDs [1]. Multiple genomic mutations contribute to these defects, but it has not been possible to estimate susceptibility based on individual variants with existing models. Human pluripotent stem cell (hPSC) derived RosetteArrays are a standardized and quantifiable model of neural tube closure and enable modeling of discrete central nervous system regional identities, i.e., forebrain vs spinal cord [2]. RosetteArray screens of CRISPR edited hPSCs containing clinically associated genetic mutations within either the folate metabolism or planar cell polarity pathways demonstrate quantifiable NTD-like phenotypes. Here, we present the platform's ability to quantify and model a clinically relevant multifactorial NTD scenario where the folate pathway mutated hPSC line demonstrates an increased sensitive to folate pathway inhibitor Methotrexate, when compared to its isogenic control. Additionally, we will present the planar cell polarity pathway mutated hPSC line's spinal region dependent phenotype, demonstrating the importance of regional identity when modeling human NTD risk. These preliminary results demonstrate the RosetteArray's ability to model and quantify NTD susceptibility based on individual genetic variants in a human etiological background. Future work involving spina

bifida patient-derived iPSCs evokes the possibility of personalized risk assessment and the development of precision medicine treatment approaches.

References

[1] Iskandar, B. J. and Finnell R. H. (2022). *N Engl J Med 387*, 444-450.

[2] Knight, G. T. et al. (2018). *eLife 7*, e37549.

Presentation: Poster

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Integration of human stem cell-based embryoid bodies into a microfluidic multi-tissue platform for systemic embryotoxicity testing

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Assessing compound embryotoxicity constitutes a central part of every drug development process. However, current *in vitro* assays do not include complex embryo-maternal interactions during pregnancy and are mostly based on the use of murine-derived cell models, which are of limited predictive power due to considerable inter-species differences.

Here, we present a multi-organ platform, which combines a microphysiological model of the placental barrier with 3D embryoid bodies (EBs), derived from human induced pluripotent stem cells (hiPSC). The platform consists of two independent fluidic networks, representing maternal and embryonic blood circulation. Both fluidic networks are separated by a semipermeable membrane, which serves as a scaffold to form and culture a human placental-trophoblast barrier in the maternal culture compartment. The hiPSC-derived EBs are cultured in immediate vicinity to the placental barrier in a hanging drop on the embryonic side, which enables direct interaction and molecule exchange between the tissue models through the liquid phase.

In a first step, we successfully established the formation and cultivation of hiPSC-derived EBs in our microfluidic device and compared their growth behavior and morphology to those achieved with standard well plates. To evaluate toxicity effects on embryonic development, we established an optical clearing method to visualize the spatial distribution and differentiation of hiPSCs into derivatives of the three germ layers. We further developed a qPCR-based panel of genes, expressed during early embryonic development, to evaluate altered gene expression patterns in differentiating EBs. In a next step, we integrated the placental barrier into the system and confirmed hEB growth and differentiation under co-culture conditions. These results show the potential of the platform to mimic physiologically relevant conditions on chip and lay a promising foundation to study the effects of compounds at the embryo-maternal interface in an entirely human-based system.

Presentation: Oral

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Human gastric extracellular matrix as a Matrigel alternative for gastric organoid culture

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Tissue-derived human gastric organoids (HGOs) are three-dimensional (3D), spherical, multicellular cultures. HGOs express several gastric epithelial cell types, form a polarized epithelium, and can be derived from biopsies for patient-specific studies, making them a more physiological experimental model than traditional cell culture. Their 3D structure must be supported by an extracellular matrix (ECM) component, most commonly, Matrigel: a laminin and collagen rich mixture derived from the Engelbreth-Holm-Swarm mouse sarcoma. As extracellular signals for growth and development can be tissue-specific, species and tissue-matched ECM for HGO culture would improve their relevance as a microphysiological system. Decellularized tissue has been well studied clinically for wound healing applications, but more recently, its function for tissue-specific ECM materials for organoid culture is being explored. Here, we describe a method for the decellularization of human gastric tissue and reconstitution of the solubilized ECM components into a hydrogel for HGO culture. Gastric tissue was obtained from organ donation or sleeve gastrectomy, then cellular components were removed using water and the detergents triton X-100 and sodium deoxycholate. The decellularized tissue was lyophilized, then homogenized and digested with pepsin to form the hydrogel solution. HGOs were passaged into human gastric ECM or Matrigel, then their growth was measured over five days of culture. After passaging, HGOs in gastric ECM re-formed less efficiently than in Matrigel, but their growth and size was comparative. To our knowledge, this is the first example of human organoids cultured within human, tissue specific ECM. Culture of HGOs within their tissue-specific ECM environment may enhance organoid differentiation through tissue-specific mechanical interactions and reduces the need for xenobiotic and undefined ECM materials.

Presentation: Poster

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A new oxygen-permeable material enabling cellular aerobic respiration both in static and perfusion MPS

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Oxygenation of cells *in vitro* is a fundamental issue, because sufficient oxygen supply enables aerobic respiration for efficient ATP production, leading to better reorganization of tissue structure and functionality. However, *in vitro* oxygenation, particularly in static condition, has been a kind of a blind spot and not completely been solved even now, although simple diffusion limitation by the layer of the culture medium was pointed out almost 50 years ago [1]. We are using a new oxygen-permeable material, polymethylpentene (PMP), as the bottom surface of culture plates and have been investigating its efficacy in hepatocyte culture. When prepared in a thin membrane (50 μm), the oxygen permeation becomes almost equal to that of conventional polydimethylsuloxiane (PDMS) membrane (300-500 μm). The cells can be oxygenated directly and satisfactory, avoiding the oxygen diffusion limitation. This allows cells to take aerobic respiration and the cells showed enhanced albumin production and drug metabolisms. The material does not seriously adsorb various drugs to evaluate better accurate estimation of drug metabolism profiles, as opposed to PDMS that resorbs various drugs [2]. In addition, the PMP membrane enabled simple formation of multilayered heterogenic liver tissues that can be called as "open organoid", which is better compatible with perfused MPS to address organ-to-organ interactions. As expected, this PMP membrane was also effective even in perfusion-type MPS. When the bottom surface of the micro-stirrer-driven perfusion type MPS (KIM plate) [3] was replaced with PMP membrane and used for small intestine and liver cell coculture, the functions of human hepatocytes are remarkably enhanced, and this was further pronounced in coculture with iPSC-derived small intestinal cells. These results indicate that perfusion of culture medium does not always meet cellular oxygen demand simply because the oxygen solubility of culture medium is about 1/70 that of blood. As such, the new oxygen-permeable material, PMP, is a promising material for cellular oxygenation in MPS toward better and physiological cellular functionality.

References

[1] Stevens, K. M. (1965). *Nature 206*, 199.
[2] Nishikawa, M. et al. (2022). *Front Toxicol 4*, 810478.
[3] Shinha, K. et al. (2021). *Micromachines 12*, 1007.

Presentation: Poster

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Discovery of novel anti-cancer components from celastrol derivatives based on patient-derived colorectal cancer organoids

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Patient-derived tumor organoids could be used to predict response to drugs accurately and help discover novel anti-cancer ingredients. Celastrol (CEL) is a natural friedelane pentacyclic triterpenoid isolated from *Tripterygium wilfordii*, which exhibits anti-colorectal cancer potential. The aim of this study was to modify the structure of CEL and evaluate CEL derivatives with colorectal cancer organoids by CellTiter-Glo Chemiluminescent cell activity detection so as to find novel anti-cancer compounds. Firstly, CEL microbial transformation was performed by *Streptomyces olivaceus* CICC 23628. Two new friedelanes derivatives (CEL-1 and CEL-2) as metabolites were isolated, and CEL-2 showed significant inhibitory activity against CCOs (IC50 = 12.9 μM, n = 4). Secondly, we synthesised a series of celastrol derivatives and find compound 4m, the most active derivative, could suppress activity of CCOs (IC50 = 132.9 μM, n = 4). Especially, they both showed much better activity than the positive drug oxaliplatin (IC50 = 75.5 μM, n = 4). Finally, we used a colorectal normal organoid to evaluate the toxicity of compounds to normal tissue. CEL-2 (IC50 = 661.3 μM) showed higher safety than 4m (IC50 = 8.459 μM). In conclusion, Drug screening based on organoids is a fast and effective method to find novel anti-cancer components. CEL-2 is expected to develop as a potent anti-tumour agent from celastrol derivatives.

Presentation: Poster

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Endothelialization of bifurcating microchannels for 3D tissue models

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Compared to two-dimensional cell culture, 3D tissue models enable biomedical research in more physiologic-like conditions. However, fabrication of functional 3D tissue models remains challenging due to the need of vascularization. Here, thermoresponsive poly(2-oxazoline)s (POx) were used to fabricate sacrificial microfilamentous scaffolds, which were embedded into cytocompatible hydrogels using dedicated perfusion chambers. Upon scaffold dissolution by temperature lowering, perfusable bifurcating channels were created. Micronetwork endothelialization efficacy with human umbilical vein endothelial cells (HUVECs) was determined by advanced microscopic techniques and monolayer functionality was evaluated using barrier assay and stimulation with inflammatory cytokine TNF-α.

When the generated channel was seeded with HUVECs, a rapid cell attachment and formation of functional monolayer occurred within 3 days. The diffusion of FITC-labelled dextran (200 kDa) in endothelialized samples was reduced by nearly a half after 2 h compared to non-endothelialized ones. TNF-α stimulation in endothelialized samples exposed to bidirectional flow on the rocking shaker resulted in upregulation of VCAM-1 and E-selectin, as compared with non-perfused microchannels. The interactions of the endothelial monolayer with cells embedded in the surrounding hydrogels are currently being investigated.

Taken together, the developed vascularized 3D models with functional endothelial monolayer can serve as a tool to investigate cell activation and angiogenesis, macromolecular, nanoparticulate or drug transport, as well as serve as a building block for generation of larger tissue constructs.

Funding: This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 326998133 – TRR 225 (subprojects B02, B08, and A06).

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Impact of microfluidic systems on 2D and 3D cell cultures

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Over the last several decades a number of research teams developed advanced *in vitro* cell-based systems that try to better mimic the physiological conditions of animal and human cells and organs *in vivo*. This has led to the evolution from planar cell culture (2D) to cell culture on 3D scaffolds, and the incorporation of cell scaffolds into microfluidic devices, which is an important step in a trend toward better biomimetic tissue models. Microfluidic systems have become a valuable tool to increase the physiological relevance of 3D cell culture by enabling spatially controlled co-cultures, perfusion flow, and spatial control over signaling gradients [1,2].

The presented study focuses on the development and validation of methods applicable to the biocompatibility testing of medical devices. In our work, we have evaluated the effect of the static and dynamic conditions on the cell culture using mouse fibroblasts 3T3 cell line (used by ISO 10993-5) and on the 3D reconstructed human tissue models of cornea and skin. Dynamic conditions were induced by the fluid-dynamic bioreactor (MIVO®), allowing us to mimic the flow dynamics of living organisms. Using 3T3 cells, we have observed changes in early cell adhesion, metabolic activity, and proliferation induced by the transition from static to dynamic conditions. Various dynamic conditions were evaluated differing in the flow rate and media. A similar experiment was conducted with 3D tissue models.

The pilot study showed differences in the cell shape and orientation (toward the flow). In the case of the 3D models, the media circulation led to slight metabolic and morphological changes. These findings are useful considering their application in tissue engineering in order to mimic as close as possible the physiological conditions for instance in the cardiovascular system.

This work was supported by the APVV project APVV-19-0591 and VEGA 2/0153/20

References

[1] van Duinen, V., Trietsch, S. J., Joore, J. et al. (2015). Microfluidic 3D cell culture: from tools to tissue models. *Curr Opin Biotechnol 35*, 118-126. doi:10.1016/J.COPBIO.2015.05.002

[2] Castiaux, A. D., Spence, D. M. and Martin, R. S. (2019). Review of 3D cell culture with analysis in microfluidic systems. *Anal Methods 11*, 4220-4232. doi:10.1039/C9AY01328H

Presentation: Poster

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A liver and testis multi-organ-chip: Towards a systemic male reprotoxicity model

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Current benchtop reprotoxicity models typically do not include hepatic metabolism and interactions of the liver-testis axis. However, these are important to study the biotransformation of substances.

In this study, Testicular organoids derived from primary adult testicular cells and liver spheroids were co-cultured in a multi-organ-chip circuit for over a week. Additional single-organ-chips and well plates (static) were loaded only with testicular organoids or liver spheroids for comparison. Subsequently, the system was challenged with cyclophosphamide, an alkylating anti-neoplastic prodrug that has demonstrated germ cell toxicity after its bioactivation in the liver, to replicate the systemic human liver-testis interaction *in vitro*. Single chip-based testicular organoids were used as a control.

The Co-culture experiment revealed that the specific medium was able to maintain the metabolic activity of the tissues. Additionally, the testicular organoids developed optimally and generated higher inhibin B values, though the testosterone levels were not as high as in the static culture with the testicular organoid-specific medium. By comparison, testosterone secretion by testicular organoids cultured individually on multi-organ-chips reached a similar level as the static culture at Day 7. This suggests that the liver spheroids have metabolised the steroids in the co-cultures, a naturally occurring phenomenon. The addition of cyclophosphamide led to upregulation of specific cytochromes in liver spheroids and loss of germ cells in testicular organoids in the multiorgan- chip co-cultures but not in single-testis culture.

This co-culture model responds to the request of setting up a specific tool that enables the testing of candidate reprotoxic substances with the possibility of human biotransformation. It further allows the inclusion of other human tissue equivalents for chemical risk assessment on the systemic level.

Presentation: Oral

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Microfluidic system for automated cellular perfusion: Screening compounds on monoamine transporters

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Neurons propagate signals by releasing neurotransmitters in the synaptic cleft. This action is terminated through the reuptake of molecules such as dopamine, serotonin and norepinephrine carried out by membrane transporters. The monoamine transporters are of great interest for their role in physiological activity of the body and their link to mental and behavioral disorders. The characterization of compounds on neurotransmitter transporters helps obtain useful information on the molecular mechanisms of the transporters. Nowadays the well-established static well-plate assays are used to study the interaction of the compound with the transporters but still suffer from some drawbacks, e.g., lack of automation, need for human operations, and risk of contamination due to repeated actions. This work describes the development of a novel automated microfluidic system for the characterization of compounds on monoamine transporters.

A microfluidic system was built and optimized to perform release assays to study monoamine transporter-mediated substrate efflux. The platform was assembled using a pressure-driven flow controller, flow sensors, and valves (Elveflow), and optimized for the automated perfusion of 12 channels simultaneously on a microfluidic chip. The system was demonstrated to enable precise flow control providing an equal flow rate in each channel through the integration of sensors equipped with feedback control. D-Amphetamine and p-Chloroamphetamine were used as releasing agents for hNET, and hSERT, respectively, to validate the system, and both induced efflux, while use of inhibitors GBR12909 for hNET and paroxetine for hSERT inhibited efflux, consistent with literature [1]. The current proof of concept platform maintains a small footprint to minimize the equipment needed and has the potential to obtain a higher-throughput by using all available channels of the pressure controller in order to scale up the system and increase the parallelization.

Reference

[1] Maier, J., Rauter, L., Rudin, D. et al. (2021). α-PPP and its derivatives are selective partial releasers at the human norepinephrine transporter. *Neuropharmacology 190*, 108570. doi:10.1016/j.neuropharm.2021.108570

Presentation: Poster

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Engineered basement membrane enhancing barrier functions of human iPS-derived blood-brain barrier model

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The basement membrane (BM) of the blood-brain barrier (BBB), a thin extracellular matrix (ECM) sheet supporting brain microvascular endothelial cells (BMECs), plays an important role in regulating the unique physiological barrier function of the BBB, which is a major obstacle to brain drug delivery. Due to the difficulties in mimicking the unique characteristics of BM on *in vitro* systems, current *in vitro* BBB models have suffered from limited physiological relevance. Here we developed a highly improved human BBB model achieved by an ultrathin ECM hydrogel-based engineered basement membrane (nEBM) supported by a sparse electrospun nanofiber scaffold that provides BMECs with an *in vivo* BM-like microenvironment. A BBB model reconstructed on the nEBM showed the physical barrier function of the *in vivo* human BBB through the ECM mechanoresponse to physiologically relevant stiffness (~500 kPa) and exhibited high efflux pump activity. These features of the proposed BBB model enable modeling of ischemic stroke and reproduce the dynamic changes of the BBB, immune cell infiltration and drug response. Therefore, the proposed BBB model represents a powerful tool for predicting BBB penetration of drugs and developing therapeutic strategies for brain diseases

Presentation: Poster

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Epithelial wrinkling and wrinkle-to-fold transition *in vitro*

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Though the folding of epithelial tissues in response to compressive forces is commonly studied using *in vivo* animal models, these models have limitations not only in observing the folding events in real-time but also in controlling experimental parameters independently. Here, we developed an *in vitro* epithelial folding model that is an epithelial bilayer composed of epithelium and extracellular matrix (ECM) hydrogel emulating *in vivo* epithelial tissue folding, which allows *in situ* real-time observation of epithelial wrinkle and fold formations. Beyond the simple folding of a single epithelium layer, we first realized a hierarchical transition of the epithelial bilayer from multiple wrinkles to a fold under uniaxial compression. Varying experimental parameters of the present model independently revealed that both the compression limit of epithelium and the poroelasticity of the ECM hydrogel in the bilayer orchestrate to determine the epithelial wrinkle and/or fold formations. The present epithelial folding model would pave the way for investigating the underlying mechanism of various *in vivo* epithelial folding to be applied to developmental biology and tissue engineering.

Presentation: Poster

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Development of organoid-derived hepatocytes and enterocytes from human primary cells, biopsy, and iPS cells for pharmaceutical research

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Microphysiological systems (MPS) are getting much more attention for predicting and evaluating drug effects. MPS having the functions of liver and small intestine is expected to reproduce the drug's ADMETox properties. The development of cellular model, such as human hepatocytes and enterocytes (small intestinal cells), is required in addition to the development of the MPS devises. Use of regenerated medicine-related techniques such as iPS cells and organoids are expected for this purpose. In this study, we have developed human hepatocytes derived from organoids generated from the iPS cell-derived hepatocytes, human hepatocytes derived from organoids generated from commercial available cryopreserved human hepatocytes, human enterocytes derived from organoids generated from the iPS cell-derived enterocytes, and human enterocytes derived from organoids generated from human small intestinal biopsy samples. These human hepatocytes and intestinal cells could passage, proliferate for a long term, be stored as a frozen cells, and showed higher hepatic or intestinal functions, respectively. When

these human hepatocytes' and intestinal organoids were cultured with differentiation medium under 2D-conditions, their hepatic and intestinal functions were dramatically enhanced. In the case of intestinal organoids, they formed a tight monolayer in 3 days when the organoids were dissociated into single cells and seeded on cell culture inserts. The drug-metabolizing enzyme activities (CYP3A4 activities) in these cells were much higher than those in conventional human iPS cells-derived hepatocytes and enterocytes, and were similar to those in primary human hepatocytes and enterocytes (or microsomes). We also generated human hepatocytes and enterocytes in which the gene-coding drug-metabolizing enzyme and transporter are modified by genome-editing technology. These lead to the tests reflecting the inter-individual differences in cytochrome P450 metabolism capacity and drug transport activity and drug responsiveness. Our research will contribute not only to produce the hepatocytes and enterocytes suitable for the *in vitro* studies using MPS, but also to dramatically improve drug discovery process.

Presentation: Poster

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Development of a testis tissue culture device for drug toxicity testing

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Anticancer drugs can cause impaired spermatogenesis in immature testis tissue due to side effects [1]. In drug development, *in vitro* testis tissue culture is investigated as a method to evaluate the toxicities of anticancer drugs on spermatogenesis, in addition to animal experiments. *In vitro* testis tissue culture methods allow observation of spermatogenesis by culturing testicular tissue removed from mice on agarose gels soaked in a culture medium [2]. The polydimethylsiloxane (PDMS) chip prevents degeneration and necrosis of the central portion of the tissue, which is possible by controlling the thickness of the testis tissue and enables stable tissue culture for spermatogenesis [3]. When observing the tissues in this culture method, a stereomicroscope is used. However, detailed observation of spermatogenesis is difficult with a stereomicroscope, so observation with an inverted microscope is necessary. We have developed a testis culture device that enables high-resolution observation of spermatogenesis using an inverted microscope for application to toxicity testing.

The testis culture device consists of a PDMS bottom well plate and a porous membrane chip that enables thickness control of testis tissues. Testis tissue is placed on the PDMS bottom, covered with the porous membrane chip, and a culture medium is introduced into the well. This method allows testis culture to be performed within the focal distance of an inverted microscope.

We compared our proposed method with a conventional *in vitro* testis tissue culture method to verify whether testis tissue from transgenic mice (Acr-GFP), in which spermatogenic cells are labeled with a green fluorescent protein, can be used for high-resolution observation of spermatogenesis. As a result, Acr-GFP expression was confirmed in the proposed method conventional method and conventional method, and the proposed method enabled more detailed observation of testicular tissue. These results indicate that high-resolution observation of spermatogenesis is possible using the device. We can conclude that the device can be used to evaluate the effects of exposure to anticancer agents on spermatogenesis in detail.

References

[1] Delessard, M. et al. (2020). *Int J Mol Sci*.
[2] Sato, T. et al. (2011) *Nature*.
[3] Kojima K. et al. (2018). *Biotech Bioeng*.

Presentation: Poster

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Tumor-on-chip to evaluate CAR-T-cell based cancer immunotherapy efficacy *in vitro*

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The treatment of patients with solid tumors in terms of CAR-T-cell immunotherapies is not very effective to date and there is still a lack of understanding the mode of action and side effects. To overcome these obstacles of cell-based cancer immunotherapies, we need physiologically relevant test systems that can mimic the patient's tumor and its complex microenvironment.

For this aim, we established a tumor-on-chip-system which serves as an *in vitro* test platform for CAR-T-cell products. The microfluidic platform can hold tumor models of different origins like tumor spheroids generated from cell lines, tumor organoids or even patient-derived microtumors (PDMs). The platform consists of two polydimethylsiloxane layers sandwiching a porous PET membrane. It contains six tumor chambers to house the cancer model embedded in a hydrogel. On chip, nutrient and oxygen supply is provided via the perfusion channel lined with endothelial cells, which also allows for perfused CAR-T-cell administration

and their migration through a physiological barrier. CAR-T-cell trafficking to the tumor-site can be traced microscopically.

In this study, PDMs isolated from fresh primary tumor samples (e.g. cholangiocarcinoma, breast carcinoma, glioblastoma, uveal melanoma) served as a tumor model in the tumor-on-chip platform. As T-cell based immunotherapy, CAR-T-cells targeting CD276 and their tumor killing efficacy were evaluated. Therapeutic CAR-T-cell responses were monitored microscopically by means of PDM viability and using an LDH-release based cytotoxicity assay. Efficient CAR-T-cell products were identified by a significant increase in cytotoxic microtumor killing and CAR-T-cell induced tumor apoptosis was quantified by M30 ELISA assay. Furthermore, CAR-T cell activation as a result of target antigen recognition during on-chip co-culture was monitored by quantification of IFN-gamma release from the perfused media.

Our tumor-on-chip system encompassing different tumor models and a vasculature-like perfusion enables the study of immune-tumor cell interaction and provides a new platform for preclinical cancer immunotherapy studies. Moreover, it may facilitate the further implementation of quality control procedures into the production process of cell-based cancer immunotherapies.

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Bioengineering investigation of multiple organ-on-chip platforms with an advanced 3D manufacturing process in the field of translational research

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For the past few years, microfluidic technologies have become a distinct new area of research enabling fluid manipulations in channels with dimensions of micrometers. These devices allow the development of organ-on-chip (OOC) systems integrating biological environments in a single chip. The idea of the investigation consists of replacing conventional microtechnological SU-8 patterned mold manufactured in a cleanroom with the goal of accelerating polydimethylsiloxane (PDMS) chip fabrication. The purpose of this project, on one hand, was to manufacture multiple OOC platforms using the high-resolution 3D printer Asiga MAX X27 UV with a transparent and biocompatible resin. Two methods were used for comparison: full chips for immediate use and molds as templates for microfabrication with PDMS. The polymerized resin mold, in its raw and unprocessed state, tended to leach residual monomers and photoinitiators from the resin into the PDMS molded slab. To solve the problem, a combination of temperature and UV treatment prevented this leaching [1]. With CAD designs and 3D printer parameters adjustments, the result enabled successful PDMS counterpart replications with features of 50-100 µm, such as pillars, to compartmentalize hydrogels into chips. Sterilization verification with basic and advanced cytotoxic tests was studied to ensure the seeding of various human cells and advanced cellular models. On the other hand, the efficiency and functioning of these OOC were investigated using different hydrogels containing spheroids or organoids stained with GFP and the fluorescence was visualized in the device under a confocal microscope ZEISS CD7. This alternative technique enables on-site manufacturing without the ultimate need to access a cleanroom, as well as allows the OOC to be prepared in a matter of hours instead of days by making it accessible to a community of scientists without prior microtechnology knowledge.

Reference

[1] Venzac, B., Deng, S., Mahmoud, Z. et al. (2021). PDMS curing inhibition on 3D-printed molds: Why? Also, how to avoid it? *Anal Chem 93*, 7180-7187. doi:10.1021/acs.analchem.0c04944

Presentation: Poster

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Electronic blood vessels based on microfluidics

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Microfluidics has emerged as a useful approach for the construction of microphysiological systems that can mimic the functions of the physiology and biochemistry of complex multi-cellular organisms, such as those of human beings. Increasing access to polymeric structures that can be turned into mesoscale structures using sophisticated tools of photolithography has enabled many researchers to come up with interesting tissue-like structures. Conductive inks made from lipid metals-polymer composites (MPC) can be encapsulated within microfluidic channels that house conducting wires that are flexible and stretchable. Combining MPC with our work in rolling 2D sheets into 3D structures that resemble the structures of natural blood vessels that have three layers of different types of cells, we can obtain electronic blood vessels. These blood vessels have improved endothelialization to prevent re-narrowing, a big problem for small-diameter blood vessels, as well as the ability to carry out reversible electroporation on each layer of the cells to introduce biomacromolecules controllably into the cells. These electronic blood vessels can be useful for tissue engineering, regenerative medicine and gene therapy.

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High throughput manufacturing of self-organizing organoids with good uniformity

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The organoids are miniaturized and simplified versions of organs cultured *in vitro*, and they have become one of the most promising models in the field of disease research and drug development. Currently, most operations on self-organizing organoids have to be done manually, which leads to large deviations in size, cellular composition and functions of organoids. Standardizing and automating the manufacturing of organoids with good uniformity become the key to promote the application of organoids in pharmaceutical industry.

We have successfully developed an automated organoids workstation, with which up to 50 plates (96- or 384-well) of organoids can be cultured and treated automatically. The organoids manufactured by this workstation show much smaller deviations in size, cellular composition and functional outcome. This platform enables manufacturing organoids in a large scale manner.

References

[1] Yokoyama Y. et al. (2018*). Biol Pharm Bull 41*, 722-732.
[2] Rose, S. et al. (2022). *Arch Toxicol 96*, 243-258.
[3] Lewis-Israeli, Y. R. et al. (2021). *Nat Commun 12*, 5142.
[4] Davidson, S. M. et al. (2021). *Cardiovasc Res 117*, 2161-2174.
[5] Nishimura, A. et al. (2019). *J Tissue Eng Regen Med 13*, 2246-2255.
[6] Ma, Z. et al. (2021). *Electrophoresis 42*, 1507-1515.

Presentation: Poster

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3D dynamic cultures of high-grade serous ovarian cancer organoids to model innovative and standard therapies

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Ovarian cancer (OC) is the most serious gynecological cancer that kills more than 140,000 women worldwide. It is characterized by non-specific symptoms and the absence of prevention tools and early screening tests. In more than 60% of cases OC is diagnosed late in an advanced stage and the prognosis is generally poor. In this scenario, it is necessary to develop *in vitro* models that can faithfully replicate the human tumor and develop novel personalized therapies.

Applying micro-fluidics to organoids (self-organized 3D organ-like construct that mimic the morphological and histological characteristics of the corresponding *in-vivo* organ) allows to generate powerful human models for studying organ development, diseases, and drug testing. In fact, microfluidic systems are designed to simulate the *in-vivo* environment. Moreover, it is possible, through microfluidics, to simulate physical shear stress and stretch, control the concentration of nutrients and factors in the media in order to induce biological processes (e.g proliferation, migration, differentiation, inflammation) including tumourigenesis [1,2].

Here, it has been developed a protocol to culture patient derived High-Grade Serous Ovarian Cancer organoids that replicates the patient's morphological and histological characteristics and that can replicate the patient's response to the drugs. With the aim to perform a drug screening in a passive microfluidic system, it was established the best conditions for culturing patient derived cancer organoids in the OrganoPlate® 2-lane 96 Mimetas platform. Moreover, it has been investigated the penetration of nutrients and drugs both in static and dynamic organoid cultures.

In conclusion, a protocol for culturing organoids in a passive microfluidic system with a high growth rate was developed and a pharmaceutical screening was performed on these organoids highlighting the advantages of using a compact passive microfluidic platform.

Acknowledgments: Associazione Italiana per la Ricerca sul Cancro – AIRC IG23566

References

[1] Saorin, G. (2023). *Semin Cell Dev Biol*. doi:10.1016/j.semcdb.2022.10.001
[2] Duzagac, F. (2021). Microfluidic organoids-on-a-chip: Quantum Leap in Cancer research. *Cancers (Basel)*.

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Application of hiPSC derived cardiac organoids in drug evaluation by electrophysiological test

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With the development of iPSC and 3D organoid technology, generating the self-organizing cardiac organoids from hiPSC has been available since 2021. The cardiac organoids can be used in the research of various heart diseases, cardiotoxicity testing, and the development of drugs. The excitability, automaticity and conductivi-

ty of myocardium are based on the electrical activity of myocardium, and the electrophysiological technique is an important tool to test heart functions.

We have successfully produced the cardiac organoids derived from human iPSCs with good uniformity using self-developed automated organoid workstation. The cardiac organoids have cavities, steady pulse, and the major cell types in heart such as cardiomyocytes, endothelial cells and stromal cells. The electrophysiological responses of normal cardiac organoids and those treated by different drugs are analyzed using the myocardial electrophysiological technique. The spontaneous or induced electrical activities of cardiac organoids can be tested with MEA. The patch-clamp technique was used to detect the changes of action potential during beating. And the calcium transient technique was used to analyze the changes of intracellular calcium level in cardiomyocytes. Compared with the 2D cardiomyocytes, the cardiac organoids can evaluate not only the depolarization characteristics of cardiomyocytes, but also the synchronization of depolarization signals at different loci in the organoids.

References

[1] Zhang, H. et al. (2018). *Sheng Li Xue Bao 70*, 281-286.
[2] Goldfracht, I. et al. (2019). *Acta Biomater 92*, 145-159.
[3] Nugraha, B. et al. (2019). *Clin Pharmacol Ther 105*, 79-85.
[4] Hofbauer, P. et al. (2021). *Cell 184*, 3299-3317.

Presentation: Poster

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A barrier-on-chip platform with integrated impedance measurement generated by a novel fast prototyping approach

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Impedance sensors and nanoporous membranes separating fluidic channels are key elements in organ-on-chip systems (OoCs) for *in vitro* modeling of barrier-forming tissues like the blood-brain barrier [1]. Among other fabrication methods, injection molding has emerged as a scalable fabrication technology for microfluidics, including OoCs, that can support their commercialization [2]. However, this method is unsuitable for pre-series production, as even minor design changes are costly. On the other hand, 3D-Printing can typically not be used to produce nanoporous membranes and integrated microelectrodes. Here, we report a barrier-on-chip platform with integrated impedance sensing on nanoporous membranes fabricated via fast novel prototyping, bridging the gap between idea and product.

Our platform comprises 3D-printed polyacrylate-based millifluidics with two stacked channels separated by a commercial track-etched polyester membrane (PET, 400 nm pore size), on which gold microelectrodes were structured by lift-off lithography. The membrane was cut to size with a femtosecond laser. The platform was sealed with screws and silicon O-rings to facilitate reopening for cleaning or further tissue analysis. Luer ports allow for the connection of 3D printed media reservoirs or tubing to induce flow via pumps or rockers.

Human umbilical vein endothelial cells (HUVEC) were cultured on the membrane. The next day, pumpless flow was introduced to allow polarization of cells and barrier formation. Live/dead staining revealed… which proved the cell compatibility of the system. In parallel, barrier formation was characterized via electrical impedance spectroscopy (EIS) using the microelectrodes. Self-made electronics were used to apply sinusoidal excitation of 5 mV amplitude. Two days later, an intact confluent cell layer emerged, which was confirmed by a stable capacitance value. Further cultivation resulted in increased resistance values, indicating stronger cell-cell contacts and better barrier integrity.

Our work demonstrates that the integrated EIS allows for online monitoring of confluency and barrier integrity without a microscope, which is advantageous for high-throughput drug testing. Moreover, the fabrication method developed in this work allows prototypes to be tested and adjusted before starting volume production by injection molding, which can accelerate the development and effectively reduce costs.

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Isogenic iPSC-based 3D vessel-on-chip model of CADASIL disease reveals vascular smooth muscle cell phenotypic switching upon heterocellular interaction

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CADASIL is a hereditary small vessel disease caused by NOTCH3 mutations leading to an accumulation and deposition of NOTCH3 protein around vascular smooth muscle cells (VSMCs). The major pathogenic feature of CADASIL is the progressive degeneration of VSMCs of small and middle-sized cerebral arteries leading to vascular dysfunction. Here, we established three CADASIL and isogenic gene-corrected lines from two CADASIL patients. By using a set of standard 2D assays, we observed comparable levels of NOTCH3 and contractile proteins such as SM22 between the VSMCs derived from CADASIL and isogenic iPSCs. In addition, CADASIL iPSC-VSMCs showed reduced contraction responses upon vasoactive stimulation when compared to isogenic iPSC-VSMCs while intracellular Ca^{2+} release dynamics where comparable. As endothelial cell (EC) expression of NOTCH3 ligands, such as Jagged-1, is required for activation of NOTCH3 in VSMCs, we recapitulated EC-VSMC interactions by using an iPSC-based engineered 3D vessel-on-chip (VoC) model. In this system, CADASIL iPSC-VSMCs showed an elongated morphology and higher proliferative behavior when compared to isogenic iPSC-VSMCs while microvascular structure was comparable between the two conditions. Immunofluorescent analysis revealed increased levels of NOTCH3 and SM22 in CADASIL iPSC-VSMCs. Importantly, we confirmed that CADASIL iPSC-VSMCs present slower intracellular Ca^{2+} kinetics upon vasoactive stimulation in the VoC. Finally, CADASIL iPSC-VSMCs morphological and phenotypical differences manifested in the VoC were rescued upon inhibition of NOTCH3 cleavage and activation with the γ-secretase inhibitor DAPT. By enabling EC-VSMC interaction in the 3D VoC model, we revealed that CADASIL iPSC-VSMCs result in profound phenotypical changes that were not evident in conventional 2D assays. This study highlights the significance of patient iPSC-based VoC for modelling CADASIL and developing therapeutic strategies.

Presentation: Poster

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Development of a lymphoid organ-chip to evaluate COVID vaccine boosting strategies

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Background: Secondary lymphoid organs provide the adequate microenvironment for the development of antigen (Ag)-specific immune responses. The collaboration between CD4+ T cells and B cells is crucial to shape B cell fate and optimize antibody maturation. Dissecting these immune interactions remains challenging in humans, and animal models do not always recapitulate human physiology. Here, we developed an *in vitro* 3D model of a human lymphoid organ. We applied this Lymphoid Organ-Chip (LO Chip) system to the analysis of B cell recall responses to SARS-CoV-2 antigens.

Methods: We used a microfluidic Chip S1® from Emulate, where the top channel is perfused with antigen (spike protein or SARS-CoV-2 mRNA vaccine), while the bottom channel contains PBMC (n = 14 independent donors) seeded at high-density in a collagen-based extracellular matrix (ECM). Immune cell division and cluster formation were monitored by confocal imaging, plasmablast differentiation and spike-specific B cell amplification by flow cytometry, antibody secretion by a cell-based binding assay (S-flow).

Results: Chip perfusion with the SARS-CoV-2 spike protein for 6 days resulted in the induction CD38hiCD27hi plasmablast maturation compared to an irrelevant BSA protein ($P < 0.0001$). Using fluorescent spike as a probe, we observed a strong amplification of spike-specific B cell (from 3.7 to 140-fold increase). In line with this rapid memory B cell response, spike-specific antibodies production could be detected as early as day 6 of culture. Spike perfusion also induced CD4+ T cell activation (CD38+ ICOS+), which correlated with the level of B cell maturation. The magnitude of specific B cell amplification in the LO chip was higher than in 2D and 3D static cultures at day 6, showing the added value of 3D perfused culture for the induction of recall responses. Interestingly, the perfusion of mRNA-based SARS-CoV-2 vaccines also led to strong B cell maturation and specific B cell amplification, indicating that mRNA-derived spike could be expressed and presented in the LO chip.

Conclusion: We developed a versatile LO Chip suitable for the rapid evaluation of B cell recall responses. The model is responsive to protein and mRNA-encoded antigens, highlighting its potential in the evaluation of SARS-CoV-2 vaccine boosting strategies.

Presentation: Oral

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Translation of DILI from regulatory animals to human using 3D liver microtissues – results of the international X-species DILI validation consortium

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Many pharmaceutical programs have been discontinued due to narrow safety margins that were not adequately predicted by animal models, leading to a loss of promising, innovative therapeutic concepts. Because species differences may skew the effective prediction of drug-induced liver injury in humans, additional translational tools are needed for internal decision-making on the fate of drug candidates. Here we propose the 3D liver microtissue MPS platform consisting of cocultures of primary liver cells, Kupffer cells, and liver endothelial cells from rat, dog, cynomolgus monkey and human to assess the translatability of liver adversities observed in preclinical animal studies. A pre-competitive industry consortium of 6 pharma and 3 technology companies was formed to qualify the 3D *in vitro* platform by recapitulating the corresponding species-specific effects observed *in vivo*. Each pharma company provided compounds to the consortium with species-specific effects, along with well documented preclinical liver pathology and DMPK properties. All compounds will be evaluated in a three-tiered process. Goal is to recapitulate the *in vivo* liver toxicology at one of the 3 levels. Tier 1: Evaluation of necrosis based on cellular ATP levels and LDH leakages. If *in vivo* necrosis cannot be recapitulated by 3D liver microtissues then Tier 2: Evaluation of species-specific liver pathological phenotypes or the DILI-specific mechanisms, like impaired bile acid secretion or reactive metabolite formation. If negative then Tier 3: DILI pathway information by transcriptomics, proteomics and metabolomics analyses and histological information through tissue microarrays. The presentation will provide a status update on ongoing studies of 40 compounds.

Presentation: Poster

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The ADME-chip: Studying different application routes on a PB/PK compliant preclinical tool

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Complex human *in vitro* ADME models involving the co-culture of key organs to mimic certain exposure routes present a challenge to establish physiologically relevant organ models as well as physiologically based pharmacokinetic (PBPK) distribution behaviour in the culture environment. In our recent study, we developed a PBPK-compliant ADME 4-Organ-Chip (Chip4) with a downscale factor of 1:100,000 of the human body. The integration of an intestinal barrier model for absorption, liver microtissues for the main metabolism, a kidney model with proximal tubular-like cells and podocytes for excretion, and neuronal spheroids as a potential target organ were optimized in the chip and co-cultured for 14 days. We exposed the Chip4 to Haloperidol, an antipsychotic medication in the butyrophenone family systemically and to Carbamazepine, a tricyclic compound with anticonvulsant properties orally with a repeated dose regime. We demonstrate uptake, distribution, metabolic capacity and toxicity assessment at an organ-specific level. We aim to develop a testing system as a potential new approach methodology for toxicological testing and to increase predictability in the preclinical stage with the multi-organ-chip platform.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreements No: 681002 and 964537.

Presentation: Poster

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Cantilever microelectrodes for the monitoring of inner electrical activity of cerebral organoids

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Cerebral organoids derived from stem cells are artificially grown miniature organs mimicking embryonic brain architecture. They show a 3D organization made of multiple neural cell types. Measuring the extracellular electrical activity of cerebral organoids with conventional planar microelectrode arrays is particularly challenging due to the spheroidal shape and 3D architecture of cerebral organoids.

In order to monitor the extracellular activity of thick spheroid-shaped samples, we developed long protruding microelectrode array, curvy and spiky cantilevers, able to penetrate in the inner regions of cerebral organoids in order to measure local extracellular potential of neurons.

A new microfabrication process has been developed on the base of standard process of planar MEAs. In order to obtain vertical microelectrodes we have used the relaxation of internal stresses of a stack of silicon nitride and silicon dioxide materials deposited over a sacrificial layer. These materials having opposite stress, tensile and compressive, the release of a clamped/free beam induces a backward deflexion of the latter. A patterned metal layer included between these stressed materials allows to obtain electrodes along the curved cantilever which rise vertically, over two hundred microns.

This new type of device has been evaluated with cortical organoids derived from hESC H9 cells. After analyzing by microscopy the effectiveness of the impalement and penetration of the protruding electrodes inside the organoids we performed spontaneous extracellular measurements. These experiments were carried out using MultiChannel Systems (MCS) amplifier system and compared with standard planar and conical MEAs. Results show typical extracellular traces and cutouts of spikes recorded with this new type of MEAs. While the planar electrodes gives no results, the conical electrode can retrieve small amplitude activities while the penetrating electrodes shows large amplitude spikes.

Presentation: Poster

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Fabrication of human organoid and organ-on-a-chip based on innervation

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Organoids and organ-on-a-chips have broad application prospects in the fields of new drug research and development, disease model, personalized medicine and manned aerospace medicine. Integration of human organ-on-a-chip is confronted with one key challenge of innervation, However, there are very few *in vitro* physiological or pathological models of neural innervation have been established. The combination of human-derived induced pluripotent stem cell (hiPSC) reprogramming technology and organoids technology to construct a new neural innervation model is a bridge to realize the transformation from animal experiment to clinical trial. We have constructed a series of multi-organ on a chip that simulating the microphysiological structure of vascularized organs, such as splenic blood sinus, microvascular tumor, and blood-retinal-barrier, etc. We also constructed three-dimensional (3D) vascularized brain organoids and fused organoids using induced pluripotent stem cells and reproduced powerful physiological and functional coupling between nerves and target tissues (blood vessels, muscles and myocardium) or target organs that combined with optogenetics, 3D and 4D bioprinting. The fabricated organoids and organ-on-a-chips are used to construct *in vitro* models of neurovascular units, neuromuscular junctions and neuromyocardial junctions, and integrated with sensors, electrophysiological stimulation and on-line monitoring for high-throughput drug screening application. These organoids-on-a-chip platforms will provide quantifiable and intuitive analysis for *in vivo* behaviors, and have great scientific significance further apply to the study of target organs tissue regeneration, disease pathogenesis, therapeutic targets and drug screening etc.

Presentation: Poster

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SmartHeart – a novel 3D *in vitro* assay for improved assessment of cardiac drug efficacy and toxicity

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According to the WHO, every year an average of 18 million people die from cardiac diseases. A substantial share of these fatalities is due to cardiomyopathies that affect people of any age. In spite of recent advances in medicine and biotechnology, the current techniques used to generate engineered tissues still require expertise and produce a limited number of tissues per batch. To address this issue, we developed a novel 3D cardiac assay that enables both the formation and maturation of ring-shaped cardiac organoids as well as the acquisition of several relevant readouts, all in a single platform. To form the cardiac organoids, cell culture plates (96WP) are coated with a 3D structured hydrogel consisting of an array of conical-shaped microwells each surrounding a central pillar. Cells seeded on top of these microstructures are guided towards the ring-shaped cavity and self-assemble into a circular organoid. Using this assay, tissues formed from a mix of iPSC-derived cardiomyocytes and fibroblasts began contracting spontaneously less than 24 hours after cell seeding. Cell contractility force, beating rate and beating amplitude were easily measured through the contraction of the central pillar of tunable stiffness. The response of these tissues to classical cardiotropic drugs (verapamil, isoproterenol, dofetilide) was investigated and agreed with the literature. The excellent optical transparency of the hydrogel renders this assay completely compatible with high resolution image-based acquisition techniques. For example, live confocal imaging with a fluorescent calcium indicator shows the expected increase in cytosolic calcium during contraction. Similarly, spatial organization of cell and tissue morphology can be observed using immunofluorescence, showing, for instance, the expected striated sarcomeric fibers of cardiomyocytes in the tissue. The contraction stress, calculated from the strain and the Young's modulus of the gel, increased over the first week, reaching a plateau at day 7, and was stable over a week after that. The SmartHeart assay thus enables the fast assembly and maturation of functional cardiac organoids and the acquisition of different relevant read-outs (beat rate, arrhythmia, contraction stress) in a single platform compatible with HTS and HCS requirements for drug screening and toxicity assays.

Presentation: Poster

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Vascularized organ-on-chip models for increased biological relevance on a high-throughput platform

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Introduction: To study both intrusion of harmful agents and access of therapeutic ones, microfluidic organ-on-chip models have been developed to replace the current standard 2D cell culture models lacking biological relevance. These new generation models are also potent alternatives for animal experimentation. However, current organ-on-chip models suffer from low throughput which hampers the test of numerous biologicals. Here, we present a standardised, high-throughput platform termed AKITA® Plate, which allows establishment of up to 96 barrier cultures on one SBS standard plate. This microfluidic plate is easy to handle, compatible for automatized confocal imaging and is able for seamless integration to industrial robotic handling systems.

Methods: Vascularized cultures on AKITA® Plate are grown on semi-permeable membranes separating the top culture chamber from a bottom microfluidic channel. Endothelial cultures can be established either with single cell type as monocultures, or with tissue specific epithelial cells or organoids grown on the top culture chamber for more fine-tuned and complex models. The structure of AKITA® Plate allows ease-of-use induction of flow with a rocker, compatible with standard cell culture equipment reducing the set-up costs. Barrier formation can be visualized on the plate with live/dead staining and assayed by measuring electrical resistance or the permeability of fluorescent probes. We have established endothelialized tissue models such as the lung-on-chip, blood-brain-barrier-on-chip, and gut-on-chip.

Results: Cultured under dynamic conditions, our vascularized tissue models showed increased barrier tightness. For tissue specific co-cultures it has been noticed that the addition of endothelium increases the barrier properties both in permeation assays as well as for functional performance in studies such as inflammation tests.

Conclusion: We introduce a microfluidic high throughput commercially available platform for the modelling of vascularized organ interfaces. Compatible with standard 96- and 384-well plate formats, our AKITA® Plates are easy to handle and compatible with automatized confocal imaging processes. Tissue models grown on AKITA® Plate can be used for compound permeation studies or personalised medicine screening in a patient-relevant manner. Additionally, our standardized and translational platform offers an alternative for animal testing, reducing the costs and time that is now used in preclinical drug testing.

Presentation: Poster

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A human bone/bone-marrow-on-a-chip approach for *in vitro* culture of human bone marrow and benchmark against clinical reality

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3D microfluidic cell culture systems (organ-on-a-chip) allow for studying human physiology at organ level. Trabecular microarchitecture of cancellous bone provides not only mechanical stability, but also a microenvironment for reticular connective tissue and vital hematopoiesis in the bone marrow (BM). In joint arthroplasty, one major clinical problem is aseptic peri-implant osteolysis due to release of metallic particles and ions. Previously, we found that the *in vitro* osteogenic capacity of bone marrow mesenchymal stromal cells (BMSCs) is impaired by *in vivo* exposure to cobalt and chromium ions in the BM. For further studies we aim to establish a human bone/BM organ-on-a-chip system to (i) benchmark it to human BM specimens in regard to bone tissue homeostasis and cellular composition and (ii) to validate the system for predicting metal-ion-induced adverse biological effects.

Primary cells (MSCs, osteoblasts and mononuclear cells) from healthy BM were isolated. Human cancellous bone was decellularized, cylindrical scaffolds were prepared, MSCs and osteoblasts were seeded and matrix mineralization was induced for five days under static conditions. Subsequently, the scaffolds were transferred to the 2-Organ-Chip (TissUse GmbH, Berlin, Germany) and BM mononuclear cells were seeded before osteoclastogenesis was induced by growth factor supplementation under dynamic culture conditions (70 µl/min) for eight days. Subsequently, the scaffold was dynamically cultivated for 21 days without further growth factor supplementation.

Lactate dehydrogenase (LDH) quantification revealed cellular integrity over the whole experimental period. Quantification of soluble bone turnover factors indicated active bone metabolism. Two-photon excitation microscopy confirmed the presence of active osteoblasts, osteoclasts and the formation of reticular fibers. Flow cytometry indicated stable T-cell and monocyte populations as well as detectable levels of hematopoietic precursor cells and B-cells. For toxicity testing, treatment with clinically relevant metal ion concentrations revealed a significantly higher LDH release compared to untreated controls. Interestingly, for the same concentrations, this effect could not be observed in a 2D MSC monoculture assay.

Developing a reliable *in vitro* system for predicting adverse effects induced by implant wear in the BM is our main focus for improving preclinical routines to keep patient safety on the highest level.

Presentation: Poster

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Dermopapilla: A self-renewing mini-organ reproduced in 3D scaffold-free spheroids

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Introduction: The hair follicle (HF) is a self-renewing "mini-organ" which undergoes to continuous cycles of growth and regression, following a precise scheme in which a complex and fine-tuned interaction of signals induces deep metabolic and morphologic changes driving a growth phase (anagen), a regression (catagen) and a final quiescence (telogen). We developed an advanced *in vitro* 3D scaffold-free dermopapilla spheroid and, thanks to its plasticity and responsiveness to external stimuli, it was possible to recapitulate a physiological dermopapilla (active growth phase), a regressive dermopapilla mirroring a forced involution model (catagen-telogen) and vascularized dermopapilla by co-culturing with microvascular endothelial cells (HMVEC).

Methods: Spheroids were produced with 5000-10,000 fibroblast (HDPF) alone or in co-culture with HMVEC (ratio 3:1) in Cnt-PR -F medium or in mix with EGM™-2MV Medium (1:3 ratio). Spheroids were left in aggregation for 4 days then they were transferred in culture plates and cultivated up to 14 days.

For the forced involution model, RTqPCR by Taqman technology was applied by monitoring FGF7 and WNT5b genes. For the vascularized dermopapilla, Immunofluorescence (IF) in whole mount was performed to quantify and localize CD31 and FN1 (Leica Thunder imager), NanoString® WNT-Codeset containing 182 genes was applied comparing to standard model.

Results: Catagen-like state (regression) induced by exposure to TGFb-1 (10 ng/ml) determined a significant down regulation of FGF7 gene expression while the further application of FGF18 (100 ng/ml) induced a down-regulation of WNT5b, suggesting follicle quiescence. ATP was found reduced in the regressive model.

In vascularized dermopapilla, vessels-like structures expressing CD31 were visible as a well-organized network inside the stromal compartment, positive for fibronectin.

Nanostring results suggest an anagen-like phase between 3 and 5 days of culture (up-regulation of anagen key promoter genes VEGF, CCND3, WNTs) and downregulation of DKK-1 and BMP-

4. The full analysis of Nanostring WNT-Codeset results is still in progress.

Conclusion: The spheroids organization mimics a miniaturized functional organ providing the guidance for cells orientation, polarization, and multi-compartmentalization thanks to the crosstalk between cells.

High biological relevance and potential applications can be associated to dermopapilla models compared to *ex vivo* human hair follicle or to bi-dimensional systems.

Presentation: Poster

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High-speed volumetric bioprinting of optically-tuned bioresins into liver organoid-laden microphysiological systems

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As liver failure is a major cause for post-marketing drug withdrawal, novel *in-vitro* models are necessary to screen for markers of liver health or damage. We present an advanced *in-vitro* model using the novel, layerless, ultra-fast printing technique Volumetric Bioprinting (VBP) to create highly cellular and highly organized complex structures. An optically tuned bioresin is developed to overcome the hurdle of light scattering induced by the presence of intracellular organelles. Patient-derived liver organoid-laden structures were printed to create advanced *in vitro* models that recapitulate liver functions involved in systemic homeostasis and blood detoxification.

An optically optimized resin was achieved by combining a gelatin-derived bioresin with high concentrations of liver organoids ($> 10^7$ cells/mL) with increasing concentrations of iodixanol. Organoid viability and hepatic differentiation post-VBP were compared to extrusion bioprinted (EB) constructs. Complex organoid-laden architectures with different structural properties were printed and cultured under dynamic perfusion to evaluate the rate of ammonia metabolism.

VBP-printed 3D constructs achieved 93.3 ± 1.4% viability with intact morphology post-printing and the highest resolutions reported to date (41.5 ± 2.9 mm positive and 104.0 ± 5.5 mm negative features) compared to extrusion-bioprinted structures. There, shear forces resulted in organoid fragmentation and lower viability (73.2 ± 1.2%). Complex structures with different pore architectures were printed within 20s and showed tunable permeability and surface-area-to-volume ratio. When cultured in a dynamic perfusion system, these structures exhibited shape-dependent changes in the bioactivity of the embedded organoids and in their ability to detoxify ammonia, a blood-transported byproduct of physiological protein metabolism.

This study demonstrated the contactless bioprinting of complex biological structures (liver organoids) via VBP. Via refractive index-matching, an optically-tuned gelatin-based resin enabled high-resolution printing of cell-laden structures, obtaining previously unattainable feature sizes. Organoids displayed high viability, superior to what was found after extrusion printing, as well as hepatic differentiation capacity post-printing. Convoluted VBP-printed structures were dynamically cultured and demonstrated shape-dependent organoid function. The combination of organoid technology with the ultra-fast printing times and design freedom offered by VBP shows promise for the development of more predictive platforms for *in vitro* disease modeling and drug screening.

Presentation: Poster

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Automation for organoid assays – An integrated system with high-content imaging

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Three-dimensional (3D) cell models that represent various tissues are being successfully used in drug discovery and disease modeling to study complex biological effects and tissue architecture. However, the complexity of 3D models remains a hurdle for their wider adoption in research and drug screening. It is a long and labor-intensive process, yet, by implementing high-throughput screening and automated processing, there can be a significant reduction in the time and effort involved. Here, we describe 3D organoid culture automation that includes monitoring, maintenance, and characterization of organoids.

This method allows high-throughput compound testing and evaluation of toxicity effects. Advanced image analysis and 3D reconstruction allowed for complex phenotypic evaluation of organoid structures, including organoid size and complexity, cell morphology and viability, and the presence of differentiation markers. Also, the concentration-dependent toxicity effects of organoids in response to several anti-cancer compounds were also evaluated.

We demonstrate here the tools for increasing throughput and automation of organoid assays and compound screening and propose analysis approaches and descriptors that allow gaining more information about these complex models.

Presentation: Poster

[2] Borrill, A. J., Reily, N. E., Macpherson, J. V. (2019). Addressing the practicalities of anodic stripping voltammetry for heavy metal detection: A tutorial review. *Analyst 144*, 6834-6849. doi:10.1039/c9an01437c

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Copper ions monitoring in cell culture media via anodic stripping voltammetry: From Transwell® to organ-on-chip systems

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Ions monitoring in real-time directly in cell culture systems and in organ-on-a-chip platforms represents a significant investigation tool to understand ion regulation and distribution in the body and their involvement in biological mechanisms and specific pathologies. In this context, copper is an essential trace element whole concentration can be linked to specific pathologies, and therefore it is important to develop reliable analytical techniques in cells systems [1].

Electrochemical methods are cheap, easy and fast and thus they are suitable for *in situ* and in-line analysis. In particular, anodic stripping voltammetry (ASV) is a very versatile technique for detection of heavy metals including copper [2]. The performance of anodic stripping voltammetry for copper detection in cell culture media was evaluated, and an acidification protocol was tested to improve the voltammetric signals intensity. A Transwell® culture model was used to test the applicability of the developed acidification protocol in a real Caco-2 cells system. Finally, a microfluidic device was engineered in order to enable an automatic acidification of the cell culture medium in a separate sensing chamber without affecting the cell culture environment. A custom silicon microelectrode was integrated in the sensing compartment to perform a continuous ion monitoring in real-time. The obtained microfluidic chip could be exploited to monitor the copper or different ions concentration in an organ-on-chip model; these functionalities represent a great opportunity for the strategic high-throughput experiments demand on biological systems, with conditions close to those *in vivo*.

References

[1] Bost, M., Houdart, S., Oberli, M. et al. (2016). Margaritis, dietary copper and human health: Current evidence and unresolved issues. *J Trace Elem Med Biol 35*, 107-115. doi:10.1016/j.jtemb.2016.02.006

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Development of a novel human microphysiological system-based SELEX method for robust identification of brain-targeting aptamers for CNS drug delivery

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Blood-Brain Barrier (BBB) has posed a significant challenge for the development of drugs for the central nervous system (CNS) due to its protective function which filters out foreign substances entering the brain. To address this challenge, there has been much effort to discover BBB-specific ligands for brain drug delivery. Aptamers, which are short single-stranded DNA or RNA nucleotides that forms a 3D structure and specifically bind to target molecules, have been proposed as new BBB shuttle molecules due to their practical ability over conventional molecules such as antibodies or peptides. Traditionally, aptamer screening has been conducted through the Systemic Evolution of Ligands by EXponential Enrichment (SELEX) process, using two-dimensional cell-based culture models or animal models. However, there has yet to be a clinical breakthrough due to the limitations of these traditional screening models, which do not accurately reflect the human *in vivo* environment. Here, we present a novel human microphysiological system-based SELEX (MPS-SELEX) technology for screening robust functional aptamers. A two-channel MPS lined with human iPSC-derived brain microvascular endothelial cells, interfaced with primary astrocytes, and pericytes, were utilized to reconstruct BBB MPS, providing physiologically relevant signals to cells while retaining *in vivo*-like barrier function for aptamer screening. The human BBB shuttling aptamer (hBS01) identified through five rounds of MPS-SELEX demonstrated superior BBB penetration efficacy and brain specificity compared to random aptamers in both *in vitro* and *in vivo* models, which was not achievable in traditional *in vitro* BBB models. Nanoparticles conjugated hBS01 exhibited improved efficacy in transporting protein cargo through clathrin-mediated transcytosis. The results of this study highlight the potential of the novel MPS-SELEX technology as a new *in vitro* method for

the robust identification of brain-targeting aptamers. This research can be further developed for a nanoparticle drug delivery system, which has the potential to increase its translational potential for the clinic in the drug development process.

Presentation: Poster

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Evaluation of chimeric antigen receptor (CAR)-T cell recruitment and efficacy on an organ-chip model system

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Objective: Chimeric antigen receptor (CAR)-T cells hold great promise as new cellular therapeutics for cancer as evidenced in clinical responses to hematological cancers. However, efficacy of CAR-T recruitment and killing in solid tumors remains a major challenge and there is a need to understand why. Therefore, human-centric model systems that can quantifiably screen the ability to home to cancer tissues and effectively kill cancer cells are in high demand. We recently developed a system for inflammatory immune cell recruitment on the human Colon Intestine-Chip as a model for inflammatory bowel disease (IBD). The goal of the current study was to develop a novel workflow for measuring the recruitment and killing capacity of CAR-T cells in an Organ-Chip, including development of flexible CAR-T cell administration methods.

Methods: We seeded a representative human HER2+ non-small cell lung carcinoma cell line (A549) into the top channel and human lung microvascular endothelial cells into the vascular (bottom) channel of Emulate's S1 Organ-Chips. HER2-specific or CD19-specific (negative control) CAR-T cells were perfused through the vascular channel. Total CAR-T cell recruitment from the vasculature channel to the carcinoma-containing (top) channel and CAR-T-mediated killing (e.g., caspase activity, IFN-γ production) were measured over 72 hours. Additionally, we assessed effects of variations in flow rate and administration methods that can be easily modified with the Emulate system.

Results: HER2 CAR-T cells were recruited to the top channel, which was supported by the addition of CXCL10, a chemokine known to be expressed in some tumors, and induced significant killing as measured by caspase activity compared to controls. Administration of CAR-T with immunological co-therapies could enhance this response.

Conclusion: These early findings suggest that the human-centric Organ-Chip model can evaluate the efficacy of CAR-T cell therapies and provide a system that integrates both the recruitment and killing aspects of CAR-T function. This system is further being used to monitor CAR-T cell exhaustion and efficacy of co-therapies (e.g., that might enhance CAR-T recruitment and killing).

Presentation: Poster

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Organ-on-a-chip approach for accurate phage display screening of organ-targeting shuttle peptide

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Cell-based phage display screening technique is widely used for discovering ligands that bind to a specific receptor, with potential applications in selective drug delivery. This technique has gained considerable attention in discovering novel peptide ligands that target specific vascular beds in organs. However, traditional cell-based models have been unsuccessful in peptide screening as they do not accurately replicate the unique features of a vascular bed in a specific organ. The inner surface of blood vessels is lined with vascular endothelium that is subjected to shear stress generated from blood flow, which enables the maintenance of the structural and functional features of the endothelial cells through mechano-transduction. An organ-on-a-chip that mimics blood flow in the vascular endothelium has emerged as a promising platform that provides a more accurate representation of the physiological features of an organ, such as cytoskeletal organization, gene expression, and intracellular trafficking dynamics, compared to traditional static models. Here, we demonstrate how an organ-on-a-chip approach provides a reliable and efficient way to select organ-targeting shuttle peptides. To demonstrate this, we compared phage display selections using a microfluidic blood-brain barrier (BBB) model and a static transwell-based BBB model. Interestingly, peptides selected from the microfluidic BBB model only showed superior BBB penetration function when the microfluidic models were subjected to flow. This is attributed to the influence of fluidic shear stress on cells, resulting in different phage display selections result. Glycocalyx, a dense and brush-like structure that covers the surface of cells, is known as an intermediate between the mechanical sensation of fluid shear stress and cell signaling pathways, regulating vascular permeability and molecule adhesion. We found that the brain endothelium exhibits a thicker glycocalyx layer in the microfluidic BBB model, leading to the selective transport of peptides through the endothelium. Moreover, our re-

sults confirmed that the peptides selected from the microfluidic BBB model display significantly higher brain targeting abilities *in vivo*. This study highlights the importance of using organ-on-a-chip technology for successful phage display screening and provides evidence for its potential benefits for the future application of human drug delivery system development.

Presentation: Oral

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Peripheral inflammation microfluidic model to disclose polyphenol metabolites potential

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(Poly)phenols are found in a variety of plant-based foods and have been extensively studied considering their beneficial brain-health effects. Polyphenols consumption is linked with a reduction of inflammation markers and the neuroinflammation status of the brain [1], therefore contributing to the prevention of neurodegenerative diseases (NDs). Circulating microbiota-derived (poly)phenols metabolites (PM's) are found the most abundant in the bloodstream [2]; demonstrated protective effects in different neuronal systems by the reduction of neuroinflammatory markers, and brain microvascular endothelial cells (HBMEC) protection from an oxidative insult [3]. Despite that, the cross-communication between HBMEC cells and brain immune cells, microglia, in response to a peripheral inflammatory signal remains poorly explored.

Our goal is to unravel the potential of physiological relevant microbiota-derived dietary (poly)phenol metabolites, on the modulation of HBMEC and their communication with microglia cells (HMC3) upon peripheral inflammatory stimulation (IL-1β, IFNγ, LPS, TNFα). The physiological feature to be considered is that the appearance in circulation is simultaneous for more than one PM's and accordingly a time course of increasing concentrations [4]. The use of a microphysiological system (MPS) model adapted from Raasch et al., will enable us to understand the mechanisms related (poly)phenols metabolites brain protective potential in a physiological environment, by the delivery of PM's according to their pharmacokinetic profile.

Within this physiological setup, the molecular mechanisms behind their neuroprotection will be studied, gathering more comprehensive modelling of events during neuroinflammation in the presence of controlled delivery of physiologically relevant mixtures of PM's. Unravel the mode of action of physiological relevant protective polyphenolic molecules will be the fine tuning to the real physiological conditions that they act inside the human body, and we will establish the grounds for the development of dietary products enriched in specific polyphenols.

References

[1] Carecho (2022). *Mol Nutr Food Res.*
[2] Pimpão (2015). *Br J Nutr.*
[3] Figueira (2017). *Sci Rep*.
[4] Carregosa (2022). *CRFSFS.*
[5] Raasch (2016). *Biomicrofluidics*.

Presentation: Poster

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Self-organisation of human hepatocytes into hepatic cords on a radially perfused microfluidic device

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Liver-on-a-chip devices in recent years have shown promising results as predictive human-based *in-vitro* models during preclinical studies. In this regard, this work presents an original microfluidic architecture permitting the culture and maintenance of human hepatocytes. In this designed micro-environment, the hepatoblasts self-organise and differentiate into a tissue that mimics the dimensions and hydrodynamic conditions of a hepatic cord. Reproducing the *in-vivo* hepatic micro-architecture, we have also included a space of Disse feature to increase interaction between the tissue and the medium flow. Biomarkers of hepatocyte functionality such as Albumin secretion, MRP2 staining, CFDA excretion were used to characterise the tissue during 14 days of cell culture.

Results show that in this microdevice, HepaRG-Hepatoblasts are able to successfully differentiate into the targeted hepatic cord structure and also have homogeneous albumin expression. This was not observed when loading the microsystem with pre-differentiated HepaRG-Hepatocyte or in conventional 2D cell cultures. For the first time homogeneous differentiation of bipotent HepaRG cells to hepatocytes is reported within a microfluidic device, thanks to our original architecture.

Such a microdevice shows to be a promising tool in studying differentiation of hepatocytes and the influence of drugs on hepatic cord morphology, viability and bile secretion functions.

References

[1] Lee, P. J., Hung, P. J.and Lee, L. P. (2007). An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. *Biotechnol Bioeng 97*, 1340-1346. doi:10.1002/bit.21360

[2] Nakao, Y., Kimura, H., Sakai, Y. et al. (2011). Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. *Biomicrofluidics 5*, 22212. doi:10.1063/1.3580753

[3] Boul, Manon et al. (2021). A versatile microfluidic tool for the 3D culture of HepaRG cells seeded at various stages of differentiation. *Sci Rep 11*, 14075. doi:10.1038/s41598-021-92011-7

Presentation: Poster

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A human bone-marrow-on-a-chip system for preclinical investigation of new therapeutic approaches for autosomal recessive osteopetrosis

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Sequential seeding and dynamic cultivation of human mesenchymal stromal cells (MSCs), osteoblasts (OBs) and bone marrow mononuclear cells (BMMNCs) on decellularized human cancellous bone allows to engineer clinically relevant Bone-on-a-Chip systems that enable analysis of pathomechanisms and therapies for genetic bone disorders like autosomal recessive osteopetrosis (ARO).

ARO is caused by a defect in the bone-resorbing osteoclasts and the course is lethal if the patients are not timely treated by hematopoietic stem cell (HSC) transplantation. However, this treatment harbors high risks since it relies on allografts. A solution would be the use of autologous HSCs, in which the genetic defect has been compensated or corrected by somatic gene therapy. Before somatic gene therapy becomes clinically relevant, potential adverse effects must be identified, which is the aim of this project. We plan to evaluate two non-viral gene therapy strategies using a donor-independent iPSC-derived Bone-on-a-Chip system.

For dynamic 3D long-term cultivation, we use the HUMIMIC Chip2 from TissUse GmbH. First, monocytes are seeded onto the decellularized human cancellous bone scaffold and differentiated into osteoclasts, which represent the resorbing bone compartment. MSCs and osteoblasts are subsequently seeded to the osteoclasts and osteogenically stimulated representing the bone formation compartment. In the final step, mononuclear cells are added to the scaffold to monitor possible adverse effects of the gene therapy constructs on the immunological cell population. Bone remodelling is assessed by co-registering the scaffold with a micro-CT and additionally monitored by measuring bone formation and resorption products in the culture supernatant over the cultivation period.

The dynamic long-term cultivation allows the self-organisation of the bone cells and the interaction between different cell types, thus enabling extensive preclinical testing of novel gene therapy strategies for ARO and replacing the use of mouse models that only imperfectly reflect the human situation.

Presentation: Poster

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Extracellular vesicles as a next-generation drug delivery platform in a more physiological MPS based microenvironment

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Introduction: Tumor metastases represent the main cause of mortality in neoplastic patients. Current therapeutic protocols target cancer cells, but the damage of normal cells and tissues is the main unsolved hurdle. Therefore, the development of innovative targeted therapeutic strategies with minimal side effects and systemic toxicity represents the future clinical challenge. The role of the extracellular matrix (ECM) in tumor growth and progression is gaining growing interest. In particular, an oncofetal variant of fibronectin, termed extradomain B-FN (ED-B), is highly investigated (Vaida, 2020), since it is expressed by the newly synthesized perivascular areas of the stromal matrix in a wide range of tumor types. Among novel targeting strategies, extracellular vesicles (EVs) emerged as next-generation drug delivery platforms. EVs have an intrinsic tissue-homing capability, harnessing specific barcodes to find their target both locally and systemically.

Methods: Metastatic and non-metastatic breast cancer cell lines (MDA-MB-231 and MCF-10A, respectively) will be cultured in

an alginate-based 3D matrix (Cavo et al., 2018). Taking advantage of an organ-on-chip (OOC)-based microfluidic platform (Marrella et al., 2019), we will study the expression of ED-B in a more physiological microenvironment, in presence or absence of TGF-beta treatment, a potent epithelial-to-mesenchymal inducer. We propose an innovative approach to target ED-B with a sequence-specific small peptide exposed on EV surface by click chemistry approach. Functionalized EVs will be loaded with the chemo-therapeutic molecules to be specifically delivered toward tumor sites. The 3D tumor model is also cultured physically separated from the circulatory flow, delivering the engineered EVs.

Results: We have successfully set-up EV membrane functionalization (fEVs) and paclitaxel loading (PTX-fEVs). EVs have been isolated from both erythrocytes and plasma samples from healthy donors. The expression of ED-B has been demonstrated in the OOC-based microfluidic platform above described. PTX-fEVs will be dynamically tested for their ability to target chemotherapeutic agent in cancer cells.

Discussion and conclusion: The novel fluid-dynamic bioreactor (MIVO®) device provides an *in vivo*-like environment, allowing to recapitulate the cell-cell and cell-matrix interactions occurring *in vivo*, providing a more clinically relevant model. It may represent the perfect for mimicking systemic drug administration for more predictive drug efficacy assays.

Presentation: Poster

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Laser-assisted bioprinting of iPSCs generates embryoid bodies

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3D multicellular models are important tools in neuroscience to elucidate the developmental and functional characteristics of healthy and diseased nervous tissue. Currently, several techniques such as hanging drop, agitation, microwell, and forced-floating methods are used to create multicellular aggregates [1]. However, there is an unmet need for producing spheroids with precisely defined initial conditions on a large scale.

Biofabrication technologies such as Laser-Assisted Bioprinting (LAB) are a top choice for fulfilling these gaps. LAB enables microscale cellular patterning at a high cellular density and viability for creating spheroidal constructs with appropriate biomaterials.

Here, LAB is used to pattern iPSCs to create initial embryoid bodies. To provide a suitable environment for iPSC to proliferate, collagen type I and vitronectin were used as the receiving substrate of the printing process as well as an adjuvant in the bio-ink. iPSCs showed adherence to the formulated biomaterial and maintained high viability post printing. After 5 days of exposure to iPSC maintenance medium, the embryoid bodies continually expressed Oct4, however, in a neuronal differentiation medium showed a reduced Oct4 expression.

Overall, hPSCs have been made amenable for use in LAB to produce spheroids. This approach will pave the way for deriving spheroids from human cells that are similar to different tissues to assemble circuits *in vitro*.

Reference

[1] Bang, S., Lee, S., Choi, N. et al. (2021). Emerging brain-pathophysiology-mimetic platforms for studying neurodegenerative diseases: Brain organoids and brains-on-a-chip. *Adv Healthc Mater 10*, 2002119. doi:10.1002/adhm.202002119

Presentation: Poster

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Towards the development of a functional hepato-biliary model to monitor bile canaliculi formation

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The current knowledge of how polarized cells organize into tissues is still fragmentary, and this is particularly true in the case of hepatocytes, which polarize with neighbouring hepatocytes to form structures called bile canaliculi (BC) into which are excreted bile acids, metabolites and xenobiotics. Organoid cultures of human cells has recently garnered great attention because these *in vitro* models show greater physiological relevance and better polarization when compared to classical 2D cell cultures. Currently, most *in vitro* hepatic models in the literature show BC with an enlarged cystic morphology and without connections to each other, mimicking an intermediate stage of BC formation observed during embryogenesis or liver regeneration. Full differentiation/maturation to generate multicellular constructs mimicking the functions of mature adult hepatocytes remains a bottleneck.

In order to gain a deeper understanding of the mechanisms leading to this specific polarization of hepatocytes, we have developed a parallelized on-chip method to fabricate dense arrays of hepatic spheroids to study the formation and elongation of functional bile canaliculi. In this system, spheroids are grown in 3D on arrays of adhesive disk micropatterns on glass, which allows both parallelized live imaging and high-resolution confocal fluorescence microscopy (doi:10.1039/c9bm01143a). We have performed a pan-proteomic study to compare the human hepatocyte cell line HepG2 to its sub clone HepG2-C3A which, unlike the parent cell line, is able to form active BC. This study allowed us to identify various actors of interest and in particular, the E cadher-

in as a key protein player involved in hepatocyte polarization and in the formation and maturation of BC. We have used siRNAs in the HepG2-C3A cell line to repress the expression of E-cadherin and we have shown that this protein is critical for the formation of BC. Moreover, restoring the expression of E-cadherin triggers the formation of BC in preformed spheroids. We have thus developed a new tool for monitoring and studying BC formation and plan to refine our model by adding other pre-organized cellular structures, in particular biliary epithelial cholangiocyte tubes also obtained by micropatterning (doi:10.3390/bioengineering8080112) with the ambition of generating a first hepato-biliary network.

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Multi-sensor origami platform: Custom 3D sensing

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Bioprinting is a promising technology for engineering functional tissues *in vitro* that better replicate the anatomy and physiology of the human body. Bioprinted models can be produced with high spatial resolution and control, incorporating vascular networks and matching the biomechanical properties of organs. However, acquiring functional readouts with high resolution *in situ* remains a challenge. 3D electrodes have been developed for real-time data gathering and label-free monitoring in advanced 3D cultures, but incorporating sensors into 3D bioprinted models adds an additional layer of complexity to the fabrication pipeline. In response, a new Organ-on-a-Chip platform inspired by origami art has been developed that is equipped with multiple sensing capabilities for custom 3D sensing compatible with bioprinted tissue constructs. The platform incorporates impedance-based electrodes in a 4-point configuration to measure barrier function with electrochemical impedance spectroscopy (EIS), and a 3D MEA system with custom-made positioning to record electrophysiological activity. The platform has been validated by representing and assessing the neurovascular unit (NVU) with a coculture of brain microvasculature cells and cortical neurons, but could be used with any other cell type with barrier-forming properties and electroactive cells. Overall, this new platform offers a promising solution for integrating 3D sensing technology with bioprinted models to better evaluate tissue functionality and its response to various toxicants, drugs, and chemicals.

Presentation: Poster

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HiPSC-derived BrainSpheres mimic neuropathological phenotypes of the Cockayne syndrome B *in vitro*

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Modeling human brain development, and associated diseases, is a complex and difficult endeavor. Emerging microphysiological *in vitro* models stride forward to close the tremendous gaps in human disease modeling and drug-target identification. BrainSpheres have a complex 3D cytoarchitecture and consist of multiple relevant cell types. They represent an ideal model for the reproducible investigation of the neurodevelopmental key events (KE) within a short period of time. We developed patient-derived hiPSC-based BrainSpheres, and correlating isogenic control lines, to model the neurodevelopmental KEs, and their disruption, in the Cockayne Syndrome B (CSB). CSB is a rare hereditary disease with severe neurological phenotypes, mainly microcephaly, intellectual disability and demyelination. We observed inhibited neural progenitor cell migration in our CSB-deficient BrainSpheres, which seems to correlate with the clinical microcephaly of the patients. Disrupted autophagy, through altered HDAC activity, appears to be a key player in this *in vitro* phenotype. HDAC-inhibition partially rescued the migration in the disease BrainSpheres. CSB-deficiency *in vitro* is additionally associated with altered electrical activity, and differential expression of genes related to the GABA-switch. This may hint towards the intellectual disability of CSB patients. Finally, our disease model shows hindered oligodendrocyte maturation, which can be partially rescued by HDAC-inhibition. This finding may correlate to the demyelination seen in CSB patients. In order to further expand the tunability and physiological predictivity of our models, we developed printable 3D ALG/GG/LAM-based hydrogel cultures. Both disease and healthy cell lines formed 3D neural networks within these hydrogels. With our data we add to the in-depth understanding of the CSB neuropathology. We show, that hiPSC-based *in vitro* modeling of human diseases, personalized and generic, opens new possibilities for the identification of human-relevant drug-targets.

Presentation: Poster

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Machine learning analysis of oxygen amplifies the physiological relevance and translational capacity of vascularized microphysiological systems

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Since every biological system requires capillaries to support its oxygenation, design of engineered preclinical models of such systems, for example, vascularized microphysiological systems (vMPS) have gained attention enhancing the physiological relevance of human biology and therapies. But the physiology and function of formed vessels in the vMPS is currently assessed by non-standardized, user-dependent, and simple morphological metrics that poorly relate to the fundamental function of oxygenation of organs. Here, a chained neural network is engineered and trained using morphological metrics derived from a diverse set of vMPS representing random combinations of factors that influence the vascular network architecture of a tissue. This machine-learned algorithm outputs a singular measure, termed as Vascular Network Quality Index (VNQI). Cross-correlation of morphological metrics and VNQI against measured oxygen levels within vMPS revealed that VNQI correlated the most with oxygen measurements. VNQI is sensitive to the determinants of vascular networks and it consistently correlates better to the measured oxygen than morphological metrics alone. Finally, VNQI score is positively associated with the functional outcomes of cell transplantation therapy, shown in the vascularized islet-chip challenged with hypoxia. Therefore, adoption of this tool will amplify the predictions and enable standardization of organ-chips, transplant models, and other cell biosystems.

Presentation: Poster

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AngioMT: An *in silico* platform for digital sensing of oxygen transport through vascularized organ-chips and organoids

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Measuring the capacity of microvascular networks in delivering soluble oxygen and nutrients to its organs is essential in health, disease, and surgical interventions. Here, a finite element method-based oxygen transport program, AngioMT, is designed and validated to predict spatial oxygen distribution and other physiologically relevant transport metrics within both the vascular network and the surrounding tissue. The software processes acquired images of microvascular networks and produces a digital mesh which is used to predict vessel and tissue oxygenation. The image-to-physics translation by AngioMT correlated with results from commercial software, however only AngioMT could provide predictions within the solid tissue in addition to vessel oxygenation. AngioMT predictions were sensitive and positively correlated to spatial heterogeneity and extent of vascularization of 500 different vascular networks formed with variable vasculogenic conditions. The predictions of AngioMT cross-correlate with experimentally-measured oxygen distributions *in vivo*. The computational power of the software is increased by including calculations of higher order reaction mechanisms, and the program includes defining additional organ and tissue structures for a more physiologically relevant analysis of tissue oxygenation in complex co-cultured systems, or *in vivo*. AngioMT may serve as a digital performance measuring tool of vascular networks in microcirculation, organ-chip and organoid models of vascularized tissues and organs, and in clinical applications, such as organ transplants.

Presentation: Poster

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Modulation of inflammatory bowel disease (IBD)-specific immune cell recruitment and response with anti-TNF-α therapies in the human colon intestine-chip

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Objective: Inflammatory bowel disease (IBD) is a complex inflammatory disease, for which few effective therapies exist. The goal of our current work was to show that: i) the pathogenesis of a complex, immune cell-driven disease could be captured on Emulate's human Colon Intestine-Chip model; and ii) this could be used as a novel human-centric system to support IBD drug development including anti-TNF-α antibodies.

Methods: PBMCs were perfused through the vascular (bottom) channel in untreated "resting" or cytokine-primed Colon Intestine-Chips. Clinically relevant IBD drugs, including anti-TNF-α antibodies (Humira and Cimzia), as well as dexamethasone and AJM300 (a small molecule inhibitor of the α4β7 integrin that mediates immune cell adhesion to inflamed intestinal endothelium through MAdCAM-1) were administered through the vascular channel concurrently with PBMC administration. Total cell recruitment, effluent biomarker secretion (cytokines, calprotectin, C-reactive protein) and barrier function were analyzed for 72 hours.

Results: a4b7+ "gut-tropic" PBMCs (monocytes, CD4+, CD8+ and NK cells that can bind to MAdCAM-1) preferentially adhered and transmigrated in a cytokine priming-dependent manner. These PBMCs released clinically relevant IBD cytokines and biomarkers and induced barrier disruption, a hallmark of IBD (i.e., leaky gut syndrome). Both anti-TNF-α antibodies, Humira and Cimzia, and AJM300 caused reduced PBMC recruitment, inflammatory response, and barrier disruption > 90%. Dexamethasone had more modest effects, as expected.

Conclusion: The human Colon Intestine-Chip can both model complex immune cell-driven IBD and validate effects of clinically relevant IBD drugs. This system could provide predictive validity for pre-clinical development of new IBD drugs.

Presentation: Oral

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Integrative cancer biology-on-chip: Proangiogenic pathophysiology of platelets in microcirculation modeled in a tumor microenvironment-chip

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Angiogenesis is a prime event in tumor microenvironment facilitating its growth and metastasis. However, angiogenesis is not just driven by soluble factors released from the tumor cells but there is growing evidence that platelets play an intensive role in cancer angiogenesis, growth and progression. Tumors are capable of remodeling the blood vessels and the released factors may compromise vessel barrier integrity thereby making way for blood cells such as, platelets to extravasate towards tumor microenvironment. In this study, we have engineered an angiogenic tumor microenvironment-chip (aTME-Chip) that recapitulates the convergence of physiology of angiogenesis and platelet extravasation through the tumor vessels. Since ovarian cancer is one of the most lethal female cancers with known clinical thrombocytosis, we have employed this platform to explore the relative influence of normal vs grade IV ovarian cancer patient platelets on angiogenesis on a variety of ovarian cancer cells co-cultured in the aTME-Chip. Co-culturing of ovarian cancer cells and ECs in the aTME-Chip shows systematic barrier disruption, sprouting and angiogenesis over time in the presence or absence of moving platelets. Quantification of angiogenesis indicated toward three distinct observations with our platform. First, when platelets are introduced, they significantly increase the proangiogenic activity within the tumor microenvironment. Second, the temporal dynamics of angiogenic signaling and tumor vascularization are dependent on ovarian cancer cell type and seeding load. Finally, platelets from cancer patients are typically activated and further fuel the angiogenesis within the aTME-Chip, relative to normal controls. In summary, our aTME chip enables the recapitulation and dissection of angiogenesis due to complex and combinatorial signaling arising from platelets, endothelial and ovarian cancer cells derived from normal human subjects or patients. This engineered model will find potential applications in exploring antiangiogenic targets, combined with antiplatelet and other antimetastatic targets, which adds significant strength over prior *in vivo* and *in vitro* experimental strategies.

Presentation: Poster

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Intestine-on-chip: Establishment of human intestinal organoids-on-chip for drug absorption & metabolism studies

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Introduction: Lack of efficacy and safety issues are the main reasons for drug failure during development. Pharmacokinetics (i.e. absorption, distribution, metabolism and excretion) of drugs are usually very different between laboratory animals and humans. In addition, currently available cell-line based *in vitro* models also have limitations in predicting pharmacokinetics in human. Thus, there is a need for improved preclinical models to predict drug disposition and efficacy in humans more accurately. We aim to develop a system that functionally recapitulates the human intestinal epithelial barrier, the first site of absorption and metabolism of oral drugs.

Experimental set-up: The intestinal organoid-on-chip model was developed based on the IEBC system for intestinal tissue explants [1]. Organoid lines derived from human jejunum tissues (n = 6) and ileum tissues (n = 3) were established, to include the regional differences in absorption and metabolism relevant for the compound of interest. Functionality of the model was validated for (1) barrier integrity, (2) drug transport and (3) drug metabolism.

Results: Intestinal organoid monolayer formation and polarization was evaluated by immunofluorescence. Barrier integrity was confirmed by dextran-FITC (FD4) leakage assay and atenolol/antipyrine transport. Apparent permeability (Papp) values of talinolol and rosuvastatin showed the presence of active efflux transporters MDR1 and ABCG2. In addition, detection of midazolam and 7OH-coumarin metabolites indicated functional activity of the drug metabolizing enzymes CYP3A4 and UGTs, while CYP2C9 activity was not detected.

Future perspectives: This research is part of the academic industrial consortium "Drug Disposition-On-a-Chip", which aims to develop a multi-organ-on-chip model to study pharmacokinetics *in vitro* by combining the most relevant organs in drug disposition (i.e. intestine, liver and kidney). Further testing of the reference compounds will provide more insight in the functional capacity of the systems. Once optimized and validated, the single-OoC systems will be connected through microfluidic perfusion to mimic the combined action of PK-relevant human organs and thus can advance preclinical drug development.

Reference

[1] Eslami Amirabadi, H., Donkers, J. M. et al. (2022). *Lab Chip*.

Presentation: Poster

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Design of an electrical stimulation platform for the *in vitro* maturation of human adult cardiomyocytes

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According to the World Health Organization, cardiovascular diseases are the major cause of death worldwide, causing 32% of all global death, of which 85% are due to heart attack and stroke. During myocardial infarction, a large number of cardiomyocytes (CMs) die within few hours, leading to a permanent loss of contractile cells and deep cardiac tissue remodeling [1]. While spontaneous heart regeneration is not possible and new drug treatments are under investigation, one major challenge is the lack of predictive *in vitro* cardiac tissue models for effective *in vitro* preclinical assessment of therapies. Indeed, 2D *in vitro* cell tests fail in replicating the complexity of the native cardiac microenvironment. *In vitro* 3D cardiac tissue models are the subject of multiple research works, however *in vitro* engineering of functional adult human cardiac still represents a challenge.

Herein, we developed a new 3D cell culture system, able to mimic human adult cardiac tissue by combining engineered biomimetic substrates and electrical stimulation, in order to improve human cardiac cell maturation and functionality [2]. In details, adult human cardiomyocytes (ah-CMs) were cultured for 3 days in static condition on electroconductive photo-curable polyethylene glycol diacrylate-gelatin/poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) hydrogels (PEGDA-gelatin/PEDOT:PSS), with physiological stiffness, surface coated with human cardiac fibroblasts-produced biomatrix [3]. Then electrical stimulation was activated up to day 10 in order to promote cell-cell coupling, cell alignment and ultrastructural organization. The designed electrical stimulation (ES) platform was successfully integrated into 24-wells plates by a printed circuit for multiple and controlled electrical stimulation under sterility maintenance. Compared to cells cultured in static conditions, ES ah-CMs expressed proteins of the sarcoplasmic reticulum, such as Connexin-43 and alpha sarcomeric actinin. Moreover, ah-CMs showed

anisotropic organization according to the electric filed direction and more physiological morphology and dimension. ddPCR analysis demonstrated the influence of the substrate mechanical properties on cells gene expression.

This work is supported by ERC BIORECAR project number 772168.

References

[1] Paoletti, C. et al. (2018). *Cells*.
[2] Tandon, N. et al. (2009). *Nat Protoc*.
[3] Testore, D. et al. (2022). *Front Bioeng Biotech*.

Presentation: Poster

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Preventing PDMS drug absorption through incorporation of a PDMS-PEG block copolymer and drug pretreatment

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PDMS is widely used in fabrication of microphysiological and microfluidic systems due to its beneficial properties such as gas permeability, tunable stiffness, ease of use, relatively low cost, and by being bioinert. However, the hydrophobicity of PDMS can lead to small molecule absorption and adsorption during experiments, leading to erroneous drug screening results and undermining its applications. Alternative materials, such as elastomers and hydrogels, have thus far been unable to attain all the benefits of PDMS, and conventional methods for preventing absorption such as plasma treatment and surface coating are limited due to low throughput, efficacy, and accessibility. Clearly there is an unmet need to develop a method to prevent absorption by PDMS. To meet this need, we investigated an alternative method for preventing PDMS drug absorption through the use of PDMS-PEG block copolymer and simple drug pretreatment. PDMS or PDMS-PEG samples were incubated with drug for a week, with and without 24 hour drug pretreatment, and drug absorption was measured via UV-Vis. Drugs consisted of 5 commonly investigated cardiac therapeutics. We identified the best anti-absorption condition for each tested drug. The mean drug absorbed for all drug compounds tested in their most efficacious condition was 0.36%, representing a 92% reduction in drug absorption. We also identified 2% PDMS-PEG as a one-size-fits-all approach, as using this additive alone is able to reduce drug absorption by an average of 86.7% across all drugs tested while eliminating the need for experimental optimization by the end user. We then confirmed in an engineered heart tissue platform that this method allows for more accurate drug screening compared to standard PDMS by comparing IC50 values for the drug verapamil. These intuitive, accessible, and inexpensive procedures for significantly reducing drug absorption may easily mass adopted to ensure accurate experiments using PDMS-based microphysiological systems.

Presentation: Poster

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Completion of meiosis in mouse testicular organoids with improved germ cell survival, steroidogenesis and restoration of a tissue-specific architecture

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We present a new approach to overcome the limitations of traditional testicular organoid (TO) cultures, including histological heterogeneity, germ cell loss and absence of spermatogenesis [1-3]. Agarose microwells were tested to form TOs from mouse testicular cells in a reproducible manner and focus was put on improving germ cell survival during two weeks of cell reorganization by comparing four TO growth media. Histology, marker expression and steroidogenesis were assessed by the Periodic-acid Schiff/hematoxylin stain, immunofluorescence staining and an electrochemiluminescence immunoassay, respectively. A reorganization-supportive TO medium was defined resulting in maintenance of germ cell numbers (7 ± 5%), while 34 ± 30% of TOs recreated histological resemblance to native tissue (i.e. formation of tubule-like structures and surrounding interstitium) when grown from 32 x 103 cells. At this point, only pre-meiotic and early meiotic germ cells were present. Subsequently, defined mixtures of factors were added to the TO medium and, after 7 weeks of culture, assessed for their ability to enable germ cell differentiation. In the best condition, 85 ± 7% of the TOs recreated the testicular architecture upon long-term cultivation, which also further stably maintained the germ cell numbers. Interestingly, 3 ± 6% of the germ cells were identified as elongating spermatids and were present in 44 ± 31% of the TOs. Testosterone secretion was detected throughout culture duration (3 ng/mL at day 35), indicating functional Leydig cells. To our knowledge, this is the first study fulfilling the three benchmarks of TOs, i.e. histological resemblance, steroidogenesis and

spermatogenesis. Functional TOs offer major benefits for male infertility studies, including scalability and tailorability, which are not offered by other *in vitro* testis models.

References
[1] Richer, G. et al. (2021). *Front Physiol 12*.
[2] Baert, Y. et al. (2017). *Stem Cell Reports 8*, 30-38.
[3] Baert, Y. et al. (2019). *Biofabrication 11*, 035011.

Presentation: Poster

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Improved *in vitro* models for studying diabetes using stem cells and a user-friendly microphysiological system

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There is a critical need for improved *in vitro* systems to model type II diabetes to advance therapeutic drug candidates to preclinical studies. Significant advances have been made in this direction, with human pluripotent stem cells (PSCs) holding the highest potential as a limitless source of β-cells for disease modeling and cellular therapy for diabetes. While many protocols have been published describing the differentiation of insulin-expressing cells from PSCs, it is unclear which yield the most functional cells with reproducible glucose-stimulated insulin-secretion (GSIS). In this study, we performed a side-by-side comparison between old and new generation protocols to determine which yields the most functionally mature cells. Our results showed that the clusters obtained from the newer protocols were more mature, with mono-hormonal insulin expression and robust and reproducible GSIS. Additionally, we demonstrated that cryopreserved pancreatic progenitors yield aggregates that are capable of GSIS. Lastly, our team has developed an easy-to-use, low-cost pillar/perfusion plate that does not require pumps and specialized equipment and allows for studies of inter-organ endocrine crosstalk. Overall, this platform has the potential to facilitate drug development by providing more predictive outcomes and human models for preclinical studies.

Presentation: Poster

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A high-throughput electrophysiology platform for compound screening with a peripheral nerve microphysiological system

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Microphysiological systems (MPS) have the potential to better inform preclinical stages of drug development by enabling toxicity screening with systems mimicking *in vivo* physiology. These systems are attracting attention from the pharmaceutical industry in hopes they will curb attrition rates, lower costs, and reduce reliance on animal models. AxoSim has developed an innovative MPS, NerveSim®, for screening neurotoxic compounds using an embedded electrode array (EEA) to record compound action potentials (CAPs) from peripheral nerve cultures. Bridging the gap between *in vitro* and *in vivo*, NerveSim® measures clinically relevant electrophysiological metrics using custom, automated hardware, and software.

The NerveSim® EEA platform is a custom 24-well tissue culture plate with an integrated circuit board for high-throughput electrophysiological recordings. Each well contains a cell-restrictive outer layer and a growth-permissive inner gel that guides axonal growth into a 3D nerve-like bundle. At the bottom of the inner gel are a series of microelectrodes that are used for recording or stimulation. Dissociated rat dorsal root ganglion (DRG) spheroids were cultured for four weeks to ensure growth along the EEA. A baseline electrophysiological recording was obtained four weeks before the cultures were exposed to multiple doses of Paclitaxel for seven days. After dosing, we recorded electrophysiology again to observe deviations from baseline caused by drug treatment.

Multiple NerveSim® EEA cultures were stimulated in parallel at distal sites with both a stimulation current and frequency ramp while recording the CAPs at the DRG body and axons. From these data, we collected the conduction velocity (CV), peak response amplitude (AMP), and threshold stimulus strength (TSS). Dosing with higher concentrations of PTX resulted in slower CV, lower AMP, and higher TSS compared to the vehicle control, consistent with peripheral neuropathy. Higher PTX concentrations had reduced when stimulated at distal location, confirming the axonal degeneration expected with high dose PTX. Stimulation current ramp allowed differentiation of the responses of C fiber subtypes based on the presence of activity-dependent slowing (ADS). Fluorescent microscopy confirmed that robust axonal growth was present for lower doses, suggesting that this system can detect subtle pathological changes in electrophysiology that occur before reductions in cell viability.

Presentation: Poster

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Tissue technology enables further morphologic readouts for efficacy/toxicity in early drug screening using blood-brain barrier organoids

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Complex *in vitro* models (CIVM) offer an unprecedented opportunity to support enhanced preclinical to clinical translation by generation of high-quality preclinical mechanistic data in human systems. As an example, validated *in vitro* models of the blood-brain barrier (BBB) could facilitate effective testing of drug candidates targeting the brain early in the drug discovery process. However, adapting the standard histopathology workflows to CIVM is challenging due to their small size, differences in culturing devices, and cell composition/origin compared to animal tissue evaluation. We have developed different histotechniques using BBB organoids to support model engineering, model characterization and compound distribution as well as efficacy/toxicity evaluation in early drug screening. First, our histo-embedding technique allowed immunohistochemistry- and multiplex immunofluorescence-guided characterization with spatial evaluation of cell types. Second, histo-embedding enabled spatial evaluation of compounds via Matrix Assisted Laser Desorption/Ionization mass spectrometry (MALDI MS). As a proof of concept, we assessed the penetration of different tool compounds (BKM120, Dabrafenib, Sertraline) through the BBB, and showed that MALDI MS is a potential method to assess compound uptake and distribution in CIVM. Third, to add on the standard cell death assessment (Caspase assay), our histotechniques and digital workflow using Hematoxylin and Eosin (HE) whole slide scans allowed histopathological evaluation of single cell toxicity. We developed a high resolution (40x), single cell morphologic artificial intelligence (AI) algorithm to detect necrotic/apoptotic cells on HE scans. As a proof of principle, we used Staurosporine to induce cell death in BBB organoids and validated the performance of our algorithm towards pathologists' evaluation. Compared to the 5x standard Caspase assay, our AI morphological readout adds single cell and spatial resolution, digitalization, automation, high throughput and reproducible components giving additional insights on the necrotic effect on specific cell types and mechanism of action.

Overall, our developed histotechniques allow additional readouts for assessing efficacy and toxicity in a reproducible, robust, sensitive and high-throughput manner in preclinical drug development. Our developed label-free end-to-end digital AI readout will accelerate decision-enabling experiments in preclinical drug development and in the future, can be transferred to other organoid models.

Presentation: Oral

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A patient-derived iPSC liver acinus microphysiology system as an innovative precision medicine platform for optimizing clinical trial design for nonalcoholic fatty liver disease (NAFLD)

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Nonalcoholic fatty liver disease (NAFLD) is a major health crisis that occurs in approximately 25% of worldwide population and is accompanied by an increase in nonalcoholic steatohepatitis (NASH) that can lead to cirrhosis with liver failure and hepatocellular carcinoma (HCC). There are over 250 active clinical trials worldwide for NAFLD; however, there is currently no regulatory agency-approved therapy. Thus, the implementation of a precision medicine approach for heterogenous diseases like NAFLD face key challenges including selection of optimal patient cohorts for clinical trials, the selection of patient-specific therapeutic strategies, and optimizing the utility of patient-derived preclinical drug testing models. The heterogeneity in NAFLD is based on genomic variations, lifestyle and environment that must be recapitulated in microphysiology systems (MPS) designed for mechanisms of action (MOA) studies.

In our Clinical Trials on-a-Chip (CToC) program, we have integrated several technologies that have the potential to address these challenges including: a) enrolling over 250 NAFLD patients in the UPMC Fatty Liver, Obesity and Wellness (FLOW) Clinic where we longitudinally collect extensive clinomics data and blood samples for the production of iPSCs; b) developing a 4-cell, human liver acinus biomimetic MPS using patient primary cells to recapitulate NAFLD disease progression and as a platform for drug testing; c) developing computational and systems biology models of patient RNAseq data that can predict patient-specific responses to therapeutics; d) developing patient-derived iPSCs and differentiating them into 5 key liver cells for creating patient biomimetic twins (PBTs); e) generating the patient-specific, biomimetic MPS models to reflect the heterogeneity that can be used to experimentally test computational predictions; and (f) harnessing the Biosys-

tics-Analytics Platform™ designed to access, manage, analyze, selectively share and computationally model patient-specific clinical and experimental data.

Implementation of precision medicine for heterogeneous diseases such as NAFLD face several critical hurdles. Our CToC program creates a novel platform for precision medicine that integrates clinomics and other multimodal omics, computational methods, synthetic biology, and patient specific PBTs that are implemented to predict and test patient specific biomarkers, disease mechanisms, and efficacy and safety of therapies to improve clinical trial design to more effectively guide personalized therapeutic strategies.

Presentation: Poster

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Developing ductal tissues on a chip

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Most human organs contain ductal tissues, which play an essential role in their functionality. More than 85% of all cancers occur in ductal epithelial tissues. Having accurate models that reliably recapitulate the molecular pathways and cellular and tissue architecture is paramount to devise successful therapeutic avenues. Equally having robust platforms to test medicines during pre-clinical trials is essential for translational applications. Traditional simple cell-based assays and animal models are routinely used despite these models often lacking either morphology and/or functionality of human organs. Organ-on-chip (OOC) technology allows accurate mimicking of human tissue or organ functionality in a 3D cell culture system. Despite the numerous OOCs available, platforms that mimic the ductal tissue with its correct 3D structure and micro-environment of both the ductal tissue and the surrounding tissues are not readily available. We use DLOC Biochips that are 3D ductal organ on chip devices where the epithelial tissues are grown on cylindrical ultrathin porous scaffolds incorporated into the chips. Surrounding the scaffolds are microfluidic channels allowing co-culture of stromal tissues. The scaffold's porous design allows cell-signal exchanges between the ductal monolayer and the surrounding stroma, which is vital to biomimicry.

As a proof of principle, we co-cultured healthy human mammary epithelial cell (HMEC) with a triple-negative breast cancer solid tumour cell line (MDA-MB231) inside the duct. The stroma contained extra cellular matrix with mammary fibroblasts and adipocytes differentiated from human adipose stem cells. By RNA-seq and immunohistochemistry we then showed how it recapitulates human pathophysiology. We further validated our organ on chip by carrying out drug metabolism, toxicity and efficacy studies of cisplatin, a standard anti-cancer drugs. We also compared these results to previously collected data in animal studies where MDA-MB231 xenograft were implanted subcutaneously in immunodeficient mice treated with the same drug. Taken together this data shows that our system is a robust model for ductal organs and can be used to reduce, replace, and refine animal research in basic research and in drug development.

Presentation: Poster

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Renewable oxygen electrode sensor integration within liver organoid-on-chip microfluidic device

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To address the need for new *in vitro* model of hepatotoxicity prediction, we hereby present a microfluidic system housing a compact 1mm-long liver tissue constructed from hepatic organoids/spheroids. Reaching this size is critical as it is the size of the basic functional unit of the liver – the hepatic lobule – and creates biomimetic nutrient gradients which we monitor with thin-film electrodes included in the organoid chamber. Our system sticks out in the field of toxicity screening by its ability to compact organoids into a *thick* biomimetic tissue, by its easy seeding process with *leak-free sealing* afterward, and by its electrodes that can be *renewed mid-cultured* if fouled.

The microfluidic chip is composed of a single 1000 µm*300 µm*300 µm (L*w*h) chamber, surrounded by medium channels (both 200 µm wide) separated by 5 µm slits. The chip is sealed with a glass slide on the bottom part. On the upper part, it is covered with a thin membrane of PDMS, but the organoid chamber is left open thanks to a loading through-hole in the membrane. To close this though-hole, a specifically designed third layer is placed on top. This layer is pierce in such a shape that a PDMS plug can be fitted in – or removed from – it, hence sealing – or opening – the organoid chamber below. The whole system is coated with parylene for O_2 impermeability.

The PDMS plug is designed to provide two 3-electrode sensors, one upstream and one downstream of the chamber. Indeed, after sealing, the PDMS plug becomes the upper surface of the organoid chamber and is in contact with the cells. The electrodes are patterned on the plug surface by metal evaporation using a shadowmask. The plug being removable, a new set of electrodes can be inserted if the first one is fouled.

HepaRG spheroids are loaded in the chamber. Once closed, they are cultured with flow for 7 days. Using our O_2 sensors in coordi-

nation with colorimetric hypoxia probes, we wish to determine – by the time of this conference – the minimal flow rate for which the *biorelevant O_2 gradient* can be observed along the chamber while keeping all cells alive.

Presentation: Poster

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Understanding the neuro-developmental toxicity of heavy metals through extracellular RNA communication and synaptogenesis

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Heavy metals have been identified as a cause for neurodevelopmental disabilities, including autism, attention-deficit hyperactivity disorder, and other cognitive impairments (Vrijheid et al., 2016). These neurodevelopmental disorder (NDD) diagnoses are increasing in frequency, but the cause for this is not commonly agreed on. It is suggested that environmental exposure to hazards in combination with genetical predispositions (gene environment (GxE) interactions), can contribute and explain this rapid increase in prevalence of NDDs. Many genetic risk genes were identified, and there several environmental factors may also by associated with NDD. The current developmental neurotoxicity testing paradigm is based on low-throughput and expensive animal models, also rising ethical concerns and arguable relevance.

Thus, research in these areas is facing many challenges: from the incomplete understanding of the disorders to a lack of knowledge on the environmental factors, contributing to it, to a lack of cost efficient and relevant assays. In order to tackle some of these challenges *in vitro* cell models are on the rise, showing high complexity and human relevance. One of these models is a three-dimensional (3D) brain microphysiological system which can be implemented in neurodevelopmental toxicity research.

We utilise this model to investigate extracellular RNA communication in the course of neural development and examine the disruptions heavy metals, such as lead can cause. Further, we utilise this framework of organoid development to establish an hiPSC based synaptogenesis model. To develop this model a fluorescent marker was introduced into the presynaptic gene, synaptophyisn 1 using CRISPR/Cas9 technology. This reporter line was characterized in this project and exposed to different heavy metals (arsenic, chromium, cadmium and lead as well as their mixtures) as a proof-of-principle study. In the future, additional fluorescent tag in postsynaptic neuron will be introduced. This will enable assessing synapse formation via the colocalization of these reporters in the 3D brain organoids and allow the quantification of synaptogenesis as endpoint for developmental neurotoxicity by high throughput imaging.

Presentation: Poster

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Optimization of experimental conditions to culture microphysiological kidney tubules in FCS-free media

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Introduction: To study renal drug transport and nephrotoxicity, conditionally immortalized proximal tubule cells overexpressing Organic Anion Transporter 1 (ciPTEC-OAT1) have been used widely, in 2-D cultures and in advanced *in vitro* systems [1]. Such microphysiological or organ-on-a-chip systems fit within the 3R's vision for animal-free science, however, cells used are often cultured in media containing fetal calf serum (FCS), which raises ethical, physiological relevance and reproducibility concerns [2]. Optimization of ciPTEC-OAT1 cultures on hollow fiber membranes (HFMs), thereby creating functional kidney tubules, in FCS-free media was performed in this study.

Experimental procedure: Conditions for ciPTEC-OAT1 expansion, seeding and culture on HFMs were performed as reported [1]. ciPTEC-OAT1 were transferred to and passaged in FCS-free media (Advanced DMEM + additions ± human platelet lysates (hPL)) for at least one passage prior to cell seeding. Cell culture and experiments were performed under static conditions. Endpoints were based on cell coverage (brightfield and immunofluorescence microscopy), general cell metabolism and protein content.

Results and discussion: Initial differences in cell density on the HFM, based on protein content, were evident between optimized FCS-free+1% hPL and FCS-containing cultures (8 ± 6.9 versus 25 ± 6.5 µg protein, respectively, $n = 3$; $p < 0.05$). Metabolism of ciPTEC-OAT1 was 4.3-fold reduced when ciPTEC-OAT1 were cultured in FCS-free+1% hPL versus FCS-containing media (1072 ± 241 versus 4581 ± 440 RFU; $p < 0.0001$). Therefore, further optimization of experimental conditions for FCS-free+1% hPL culture was required. This was evaluated using different cell seed-

ing densities and by varying the number of proliferation days following assessment of complete tubule formation of ciPTEC-OAT1 on HFMs (using DAPI and phalloidin immunostaining) at different timepoints in FCS-containing and FCS-free+1%hPL media.

Conclusions: This study demonstrates steps needed to transition FCS-containing to FCS-free media for ciPTEC-OAT1 cultures on a HFM-based 3D system. Future studies are directed to optimizing perfusion assays in a 3D-printed bioreactor to evaluate further barrier tightness and functionality of drug transporters in FCS-free+1%hPL media.

The collaboration project is co-funded by the PPP Allowance made available by Health~Holland, Top Sector Life Sciences & Health, to stimulate public-private partnerships.

References

[1] Jansen, J., Fedecostante, M. (2016). *Sci Rep 6*, 26715.
[2] van der Valk, J., Brunner, D. (2010). *Toxicol In Vitro 24*, 1053-1063.

Presentation: Poster

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A model for high throughput therapeutic screening of peripheral neuropathy in a human 3D nerve-on-a-chip microphysiological system

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Toxicity is a leading reason drugs are withdrawn from the market, with neurotoxicity responsible for 16%. Peripheral nerves (PNs) are particularly susceptible to off-target effects resulting in permanent sensory-motor deficits, and chemotherapy-induced peripheral neuropathy (CIPN) occurs with a 68% incidence rate and 30% retaining effects after 6 months. CIPN can also affect clinical outcomes, with 91% of cases leading to dose reduction and a 45% discontinuation rate. Preclinical animal models are historically expensive and low-throughput and have largely failed to deliver results that translate to success in the clinic. PNs, in particular, lack predictive human-relevant *in vitro* drug screening models, with less than 7% of neurological drug candidates reaching the marketplace. Microphysiological systems (MPS), including organs-on-chips, utilizing human induced pluripotent stem cell (iPSC)-derived cells to emulate specific organ systems, have emerged as promising screening platforms to bridge the gap between preclinical and clinical success.

Engineering 3D tissues relevant to the nervous system, particularly PNs, is challenging because of the complex ultrastructure and necessity of functional outputs. AxoSim has developed an all-human NerveSim® MPS, with human iPSC-derived sensory neurons and primary human Schwann cells for screening neurotoxic compounds using an embedded electrode array (EEA) to record compound action potentials (CAPs) from PNs cultures. The efficacy of this system was demonstrated by recording cultures exposed to Paclitaxel (PTX).

NerveSim® EEA cultures were stimulated in parallel at multiple distal sites with a stimulation current ramp while recording the CAPs at the cell body and axons. From these data, we collected conduction velocity (CV), peak response amplitude (AMP), and threshold stimulus strength (TSS). Histopathology shows phenotypic responses of peripheral neuropathy including decreased fiber densities and increased degenerated fibers. The ability to collect clinically relevant data is an effective tool for *in vitro* modeling of CIPN towards screening of therapeutics for neuroprotection and neuroregeneration. Current efforts are focused first on increasing the number of myelinated nerve fibers in the NerveSim® sample, and second, on quantifying the effects of well-known demyelinating compounds, such as Cuprizone, to further establish the efficacy of NerveSim® as a drug screening platform.

Presentation: Poster

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A hollow fiber membrane-based liver-on-chip model for prediction of drug transport and metabolism

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Toxicity is a major reason of drug candidates' failure in clinical development, as rodent models used in preclinical development are not predictive in terms of pharmacokinetics (PK) in humans, i.e., absorption, distribution, metabolism and excretion (ADME). In turn, primary hepatocytes can only be cultured for a short period before losing functionality. Thus, advanced *in vitro* preclinical models with improved hepatocyte-like functions are needed to faithfully predict PK/ADME of drug candidates in humans. We aim to meet this need by establishing a bioengineered hollow fiber membrane (HFM)-based human liver model optimized for drug disposition-on-chip.

To establish the model, porous HFMs were coated with L-DOPA (biological polymeric *glue*) and defined, animal-free hu-

man recombinant laminins such as laminin-332 and were seeded with cells derived from 3D human intrahepatic cholangiocyte organoid cultures capable of differentiation towards hepatocyte-like cells. Organoid-derived cells were able to adhere to HFMs and proliferate, resulting in monolayer formation. The cell-laden HFM cultures were expanded and differentiated in serum-free media enriched with specific growth factors.

In the absence of flow, we achieved hepatocyte-like functionality of liver organoid cells differentiated on HFMs. We found that hepatocyte-like HFM cells display upregulation of genes of hepatic albumin and metabolic enzymes such as CYP3A4, CYP2C9, CYP2B6, UGT2A3 and SULT1A1, as compared to differentiated standard Matrigel cultures. Moreover, confocal microscopy revealed abundant expression in cell membranes of basolateral and apical hepatic drug transporters such as MDR1, BCRP, MRP2, MDR3, MRP3, NTCP, OATP1B1 and OCT1. Functionally, we were able to confirm drug transport and phase I and II metabolism in the system by exposing cells to compounds specific for CYP3A4, CYP2C9, CYP2B6 and UGTs, followed by measuring metabolite formation using LC-MS.

Currently, we are optimizing microfluidic perfusion on cell-laden HFMs in a 3D-printed bioreactor to evaluate barrier tightness as well as to study the transport of drug candidates across cell monolayers and to examine drug metabolism in an organ-on-chip setup. In the future, our bioengineered HFM-based liver-like model will be a part of an *in vitro* multi-organ-on-chip platform that will mimic combined action of PK/ADME-relevant human organs and thus will advance preclinical drug development.

Presentation: Poster

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Microwell arrays for long-term confinement and single-cell tracking of phenotypic heterogeneity in vascular smooth muscle populations

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Atherosclerosis results from a remodelling of the vascular wall, with vascular smooth muscle cell (vSMC) phenotypic modulation being a key driver. *In vitro* studies have demonstrated the extent of vSMC plasticity, with mature, contractile SMCs rapidly transitioning into a migratory, phagocytic phenotype [1]. However, vSMC heterogeneity may be pronounced, with recent reports highlighting vSMC sub-populations as drivers of disease. To enable tracking of large populations at the single-cell level new tools are needed. Whilst many single-cell microfluidic platforms exist, the majority are for non-adherent cell types or lack sufficient confinement for long-term culture of strongly adherent, migratory cells. Therefore, we developed an array of microwells with highly cell-repellent walls and a cell-adherent base for studying phenotypic diversity in freshly-isolated vSMCs.

Microwell arrays were produced by creating a 100 µm-thick through-hole PDMS membrane [2]. When bonded to a coverslip-bottomed dish, these through-holes (100-140 µm wide) form individual culture wells. To ensure robust vSMC confinement, PDMS functionalisation was necessary. This was achieved by flood treatment of the membrane with Lipidure®-CM, whilst temporarily bonded to a slide, before conformally bonding to the coverslip. Freshly-isolated rat vSMCs (obtained by multi-stage enzymatic digestion [1]) were seeded into devices and imaged prior to inducing phenotypic modulation by serum. Individual microwells were tracked via phase-contrast imaging, apoptosis and oxLDL uptake assays, and end-point immunocytochemistry.

Single vSMCs and their progeny were successfully confined with the microwells for > 3 weeks, with the Lipidure-coated devices showing no failures across 49 1-week experiments. Phenotypic heterogeneity was first assessed by quantifying the proliferative capacity of individual vSMCs from two vascular beds (aorta, A; carotid artery, CA). Results showed the majority of viable vSMCs remain as single, undividing cells (51%, A; 78%, CA; n = 3 animals). However, a sub-population (18%, A; 5%, CA) showed high proliferative capacity (≥ 10 progeny), in line with reports that a sub-population of vSMCs may be primed for proliferation. Furthermore, results showed a statistically significant trend of increasing Dil-oxLDL uptake and increasing Galectin-3 (a macrophage marker) expression with increased proliferative capacity.

References

[1] Sandison, M. E. et al. (2016). *J Physiol 594*, 6189-6209.
[2] Hsu, C. H., Chen, C. and Folch, A. (2004). *Lab Chip 4*, 420-424.

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High throughput cardiac ischemia human-on-a-chip platform with integrated microelectrode arrays and piezoresistive cantilevers

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Hesperos, Inc. aims to minimize animal testing to improve compound testing efficiency in preclinical trials to reduce late-stage drug failure by utilizing Human-on-a-Chip technology with iPSC-derived tissues coupled to bioMEMS devices. The objective of Hes-

peros' technology is to investigate drug compound efficacy, toxicity, and mechanism of action utilizing *in-vitro* systems with an emphasis on rare diseases. Automation of electrophysiological and mechanical measurement of organ construct function is key towards high throughput screening and quality control towards regulatory acceptance. A bioMEMS Human-on-a-Chip system consisting of a cardiac and a vasoconstriction/dilation module was developed to study ischemia (0-5% O_2, 5% CO_2 concentration) induced damage to the heart and to test candidate drugs in a high throughput setting. A specialized environmental control chamber was developed to control oxygen and carbon dioxide concentrations with lower limits of 0.1%, with programmable gas profiles. The chamber was designed to accommodate hardware to measure functional readouts across 32 systems, and environmental and medium gas concentrations. Functional readouts were integrated in the form of patterned (aligned) iPSC-derived cardiomyocytes plated on microelectrode arrays (MEA), silicon cantilevers (CL) and piezoresistive cantilevers to study the impacts of ischemic damage to cardiac tissues and evaluate the effects of pharmaceutical interventions. Custom amplification, multiplexing and stimulation control electronics were developed to measure stimulation-controlled conduction velocity (CV), field potential duration, and beat frequency on up to 32 cardiac MEAs (256 channels), cardiac force measurements on piezoresistive cantilevers, and vasoconstriction in the hemodynamic construct. In the presence of oxygen starvation, ischemic damage at medium oxygen concentrations of 0-1% reduced the CV by ~80%. Reperfusion effects were identified as a critical parameter in the ischemic damage (> 75% reduction in CV) and treatment protocols demonstrated resistance to the reperfusion effects (25-80% increase in CV compared to controls). Utilizing this model, ischemic damage and potential interventions can be studied on representative human tissue analogues with sufficient throughput for the drug development process.

Presentation: Oral

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Human engineered heart model for risk assessment of cardiac arrhythmia

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Background: Cardiovascular diseases, including abnormal heart beats (arrhythmias), are number one cause of mortality and morbidity worldwide and form a huge impact on society and economy. Until know therapy for arrhythmia mainly helps to reduce symptoms but is not able to solve underlying causes. Mainly because current models are not able to recapitulate all aspects of cardiac disease and have not proven effective in extrapolating experimental findings to the patient. Therefore, there is an urgent necessity to develop improved, more predictable disease models of arrhythmia. A human engineered 3D *in vitro* model using human pluripotent stem cell (hPSC)-derived cardiac cells is a great alternative to existing models as it can mimic different facets of arrhythmia with the goal to understand the pathogenic mechanisms of the disease and develop new therapies.

Results:

1. To more closely recapitulate onset and progression of arrhythmia in patients, a spatial organization in a specific geometry is required as it predisposes to arrhythmia. We were able to develop such a 3D cardiac tissue model by engineering a bone-like cardiac tissue by utilizing PMMA molding in combination with tissue embedding into Xanthan gum and imitating the native extracellular cardiac matrix build from collagen I and III. This model mimics the source-to-sink mismatch and thus creates a vulnerable substrate for cardiac arrhythmia.
2. This tissue could be maintained in shape for more than 40 days. By comparison of 2D and 3D culture, we generated proof-of-concept that the shape can only be maintained in 3D, but not as monolayer highlighting the importance of 3D culture.
3. We developed an optical mapping technique to visualize the conduction of calcium or voltage wave propagation through the tissue.
4. Arrhythmia-like events were triggered through subjecting the cardiac tissues to Epinephrine. Amiodarone and Quinidine, which are prescribed to lower arrhythmia incidents in patients, were successful in counteracting the effects of Epinephrine.

Conclusion: Our human-based *in vitro* model mimics several aspects of the diseased human heart and will not only shed light on underlying mechanisms of arrhythmia but will be further developed into a multidisciplinary platform for risk assessment of cardiac arrhythmia.

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Development and validation of a physiological device for compartmentalized tissue barriers

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The increasing need for complex and reliable *in vitro* models able to replicate key structures/functions of specific human tissues has driven the development of new technologies, such as microphysiological systems (MPS), to properly replicate tissue microenvironment in line with the 3R principles. Anyway, MPS are still considered not standardized enough to be systematically used. For these reasons we developed a new tape-based device, named True Tissue on Platform (TToP), representing a scalable and versatile platform for replicating tissue barrier functions.

The innovative strategy is the cartridge-based design, suitable for well-established studies and 3D cutting-edge cultures. TToP allows i) real time imaging for direct evaluation of the sample; ii) easy and safe sample retrieval, iii) reuse of the biological material in sequential treatments.

The device was designed to enable cleanroom- and PDMS-free fabrication. A 350-micron, laser micromachined cartridge was positioned in an open-well static module compatible with standard 12-well plates. The fabrication procedures allowed to obtain up to 70 devices (static module + cartridge) with an easy-to-replicate protocol in a cleanroom-free environment.

The device can host microporous membranes, on which culturing Caco-2 cells, or human reconstructed 3D intestinal tissues (MatTek-EpiIntestinal™). To demonstrate the device functionality, we monitored the cell/tissue cultures by both TEER measurements and Hoechst evaluating tissue barrier functions. Finally, we retrieved the cartridge and performed confocal imaging and TEM to verify the junctional markers (HEA, JAM-A, ZO-1 and Villin).

Caco-2 growth was monitored every 2 days, showing an increase in TEER values in accordance with cell distribution, observed by bright and fluorescent microscopy. At the end of the culture, the cartridges were easily retrieved, and fluorescence microscopy showed that Caco-2 were able to form mature monolayers after 21 days. Similarly, EpiIntestinal™ tissues demonstrated comparable TEER values in TToP vs. control. Confocal microscopy and TEM confirmed the presence of brush border, tight junctions, and 3D microtissue morphology.

TToP is a promising new technology, enabling accessible and cost-effective devices for barrier tissues *in-vitro* replication, allowing a fast integration in laboratory procedures. The cartridge-based controlled retrieval allowed to perform confocal imaging and TEM without affecting the sample integrity.

Presentation: Poster

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Development of an automated laser photoablation setup for the mimicry of a peristaltic motion of the gut

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Creating complex 3D structure in bulk material is essential to better understand the interplay between biochemical and mechanical signals of mammalian cells in a 3D microenvironment. Laser photoablation is a recent and versatile technology to provide these complex on-demand structures. However, few studies have been made using this technology and most have not integrated mechanical stimulation on the resulting 3D structure. Here, we present a home-made photoablation setup with an automated control used to engineer biological 3D structure in different hydrogels and a biological application for the creation of a gut model. The device provides a high resolution of around 1 µm and speed up to 5 mm/s. Hence, we have used such technology combined with pressure controllers to develop a microfluidic platform recreating the peristaltic motion of the gut. We have photodegraded a synthetic polyethylene glycol hydrogel using a 10 kHz laser in a polydimethylsiloxane-based microfluidic chip to create a tube-like microstructure next to thin PDMS walls. Thus, this method will provide an approach for the creation of complex 3D structures in hydrogels and the recapitulation of the dynamic *in vivo* environment of the gut.

References

[1] Brandenberg, N. and Lutolf, M. P. (2016). In situ patterning of microfluidic networks in 3D cell-laden hydrogels. *Adv Mater 28*, 7450-7456. doi:10.1002/adma.201601099

[2] Rayner, S. G., Howard, C. C., Mandrycky, C. J. et al. (2021). Multiphoton-guided creation of complex organ-specific microvasculature. *Adv Healthc Mater 10*, 2100031. doi:10.1002/adhm.202100031

[3] Ahn, J., Lee, H., Kang, H. et al. (2020). Pneumatically actuated microfluidic platform for reconstituting 3D vascular tissue compression. *Appl Sci 10*, 2027. doi:10.3390/app10062027

Presentation: Poster

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Modeling inflammatory pathways associated with proteinuric kidney disease using kidney organoids

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Focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) constitute a spectrum of rare proteinuric kidney diseases that affects adults and children and is associated with widely varied clinical outcomes. These diagnoses are based on kidney tissue histopathologic patterns which fail to identify underlying pathomechanisms of disease. The inability to identify individuals who would benefit most from a particular therapeutic has profoundly affected therapeutic trial success. Our overall aim is to combine mechanistic studies from kidney organoids generated from human pluripotent stem cells with clinical, transcriptional and biomarker data from individuals with FSGS/MCD to help inform therapeu-

tic study arm assignment within NEPTUNE (Nephrotic Syndrome Study Network), a multi-site study of the NIH's Rare Diseases Clinical Research Network.

Inflammatory pathways are thought to contribute to the pathogenesis and progression of FSGS/MCD. Indeed, we recently identified a transcriptional signature of TNFα activity in a subset of affected individuals with poor clinical outcome (PMID:36442540). We used untargeted proteomics of kidney organoids to confirm the association of clinical biomarkers with TNFα stimulation and to identify additional biomarkers of potential clinical interest (doi:10.21203/rs.3.rs-2109564/v1). Now we have identified activation of IFNγ and its downstream effectors as a key prospective pathway determinant of individuals early in the disease process who develop progressive kidney disease. Biosamples from individuals with disease revealed CXCL10 as a key serum, kidney tissue and urine biomarker of IFNγ pathway activation. IFNγ pathway activation also appears to be important in a subset of individuals with FSGS/MCD.

Transcriptional analysis revealed IFNγ-treated kidney organoids expressed IFNγ pathway genes in a dose- and time-responsive manner. IFNγ pathway transcript expression tightly correlated with secretion of CXCL10 protein. Single cell transcriptional profiling demonstrated expression of IFNγ pathway genes by podocytes and tubular cells, as expected of a urinary biomarker. Further, a clinically available JAK1/2 inhibitor ameliorated markers of IFNγ-pathway activation in organoids, consistent with recent treatment success (PMID:34741283). Thus, modeling inflammatory pathways in kidney organoids expands our ability to explore clinically relevant pathomechanisms potentially involved early in FSGS/MCD and to identify therapeutics suitable for subsets of individuals that alter disease trajectory.

Presentation: Poster

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Multi-organ microphysiological system mimics glycemic control mechanics in human pancreas-liver microfluidic co-culture

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Type 2 diabetes mellitus (T2DM) is a disease affecting approximately 10.9% adults aged 20-79 years in 2021 [1]. The largest risk factor for development of T2DM is prediabetic hyperglycemia stemming from dysfunctional interactions between multiple organs and tissues [2]. Dysregulation in glycemic control governed by interactions between the pancreas and liver plays a crucial role in creating prediabetic hyperglycemic conditions.

Classical *in vitro* models are unable to mimic complex tissue-tissue interactions. These interactions can be studied using novel micro-physiological tissue models that facilitate communication between multiple organotypic tissues. Here, we present a novel multi-organ-on-chip system that establishes bidirectional dynamic tissue communication between primary human pancreatic and primary human liver tissue in glucose metabolism [3].

This microfluidic system is pneumatically actuated using a central pumping unit connected to individual tissue chambers through microfluidic channels. From this central chamber fluid is continuously pumped in and out of the tissue chambers, thereby creating a "synthetic heartbeat". Flow and mixing characteristics to each tissue chamber can be finetuned through adjustments to the dimensions and structure of the microfluidic channels, hence creating multiple flow rates from a single pumping unit.

Biological validation of the system was established through co-culture of primary human hepatocyte spheroids and intact primary human pancreatic islets. Both tissues were shown to be viable and phenotypically relevant. Furthermore, we were able to induce insulin secretion and activate hepatic insulin response dynamics within physiologically relevant timeframes through introduction of glucose to the system.

References

[1] International Diabetes Federation. IDF Diabetes Atlas (2021). https://www.diabetesatlas.org/en/resources/

[2] Stefan, N. et al. (2016). Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes Endocrinol 4*, 789.
[3] Keulen, J. et al. (2022). Bioengineered pancreas-liver crosstalk in a microfluidic coculture chip identifies human metabolic response signatures in prediabetic hyperglycemia. *Adv Sci 9*, 34.

Presentation: Poster

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Application of a human *in vitro* testing battery for endocrine disruptor (ED)-induced developmental neurotoxicity (DNT) to refine EDC risk assessment

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Endocrine disrupting chemicals (EDCs) have been intensively studied regarding their harmful effects on human brain development. Despite increasing evidence that early developmental EDC exposure causes developmental neurotoxicity (DNT), EDC risk assessment does not feature endocrine disruption (ED)-mediated DNT endpoints. Therefore, the incorporation of ED-DNT testing strategies into the regulatory guidelines is urgently required. Currently, the identification of chemicals causing adverse neurodevelopmental effects is solely based on animal studies. However, insufficient test throughput, species differences, and ethical concerns demand alternative *in vitro* models with high predictivity for humans. Therefore, a DNT *in vitro* testing battery has been assembled including a multiplexed high-content assay based on human neural progenitor cells (hNPCs), the Neurosphere Assay.

To identify hormone-sensitive neurodevelopmental key events for ED-DNT *in vitro* assays development, we investigated the effects of hormone receptor modulation on neurodevelopmental processes represented within the Neurosphere Assay, including neural progenitor cell (NPC) proliferation, migration, and terminal differentiation into neurons and oligodendrocytes. Strikingly, oligodendrogenesis proved to be the most sensitive endpoint, being influenced by activation of liver X receptor (LXR), retinoic acid receptor (RAR), peroxisome proliferator-activated receptors (PPARs), progesterone receptor (PR), and vitamin D receptor (VDR) signaling. By incorporating both male and female NPCs in our testing strategy, and comparing the effects on human and rat NPCs, we identified both sex- and species-specific dependencies of hormonal activity during brain development. Since hormone-regulated neurodevelopmental processes provide putative targets for EDCs, the established ED-DNT *in vitro* assays will be used to screen libraries of known and putative EDCs and identify ED-induced DNT. Finally, their integration into EDC risk assessment is of highest importance for several stakeholders including regulatory bodies, industry, and the civilian population.

Presentation: Oral

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Facilitating combination therapy studies in patient-derived 3D tumour models

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Combination therapies are superior to monotherapy for treating many cancers. Further, an increasing interest in precision treatment of cancer patients has highlighted the need for microfluidic technologies capable of maximising the generation of 3D tumour models from the small cell quantities contained in needle biopsies.

We have developed a versatile MPS platform for high quality and multiplexed efficacy screening assays on spheroid co-cultures, organoids and primary tumour fragments (tumoroids). When using cell suspensions, hundreds of 3D models are created within 24-48 hours within a microfluidic cell culture array. When using tumoroids, these are seeded directly into the array according to their size. The platform is designed for self-generation of multiple drug concentration gradients, offering a unique system to miniaturize drug combination studies using patient's tissue and, at the same time, creating cost-effective and fast immune-oncology assays. Readouts, such as the model volume, phenotype and viability, are generated from image analysis prior to tissue retrieval, using epifluorescence or confocal microscopy.

Our system has been validated using a variety of cell sources. As examples of diverse and customisable screens: 1) human prostate biopsies were grown for the screening of clinical therapies on thousands of 3D multicellular structures [1]; 2) 3D co-cultures of several cell types were optimised in our platform to mechanistically study responses of the tumour microenvironment [2]; 3) CAR-T cells were used to assess their target specificity and cytotoxicity in 3D tumour-stroma co-cultures [3]; 4) ovarian tumour tissue fragments and colorectal biopsies were cultured for precision medicine studies.

These examples show the screening capabilities of our technology and especially its potential for extensive drug combination studies and precision medicine applications. Ultimately, the power of

miniaturising combination studies on tumour microenvironment-(TME) relevant patient derived models have significant opportunities to produce faster and better preclinical data.

References

[1] Paterson, K. et al. (2022). *IEEE OJEMB 3*, 86-95.
[2] Kay, E. et al. (2022). *Nat Metab 4*, 693-710.
[3] Mulholland, T. et al. (2018). *Sci Rep 8*, 14672.

Presentation: Poster

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Highly permeable nanofibrous microwell array for generation of uniform and mature kidney organoids

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Kidney organoids have received considerable attention for drug screening and disease modeling. As kidney organoids have received enormous interest as next-generation *in vitro* kidney models, various differentiation protocols for generating kidney organoids from induced pluripotent stem cells (iPSCs) have been established. Although the types of growth factors to be added are different for each protocol, most protocols are commonly based on extracellular matrix (ECM) hydrogel, where cells spontaneously assemble and differentiate into kidney organoids. While these conventional methods enabled the differentiation of iPSCs into kidney organoids, they have limitations for utilizing kidney organoids in real-life applications. For example, the low maturity of kidney organoids limits their efficacy in kidney disease modeling and cell therapy. Furthermore, these methods which result in random display and physiological irregularities of kidney organoids have a relatively poor throughput and reproducibility, limiting utilizing kidney organoids as *in vitro* drug screening models.

Here, to address the unmet need to produce mature and uniform organoids, we developed microwell arrays that enabled providing *in vivo*-like microenvironment. Considering that the kidney is highly vascularized and thus facilitates the transportation of oxygen and growth factors to neighboring renal tissues, our approach was the fabrication of a highly permeable nanofibrous (NF) microwell array, which could provide a high flux of substance like *in vivo* nephron vascular. To realize the NF microwell array, we developed a thermoforming process of NF membranes. The micro-structured NF microwell array could generate morphologically and structurally uniform kidney organoids within individual NF microwells. Moreover, we demonstrated that the high flux of substance provided by NF microwell arrays promoted the gene expression in podocytes and tubular epithelial cells and reduced nephron progenitor cells, highlighting that the NF microwell arrays could generate not only uniform but also mature kidney organoids.

Presentation: Poster

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Multiorgan-on-a-plate microphysiological platform based on a modular organ-on-a-chip device to address the microbiota-gut-brain axis

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Gut-brain axis plays an important role in maintaining systemic homeostasis through multiple signaling routes leading to the modulation of both enteric and nervous activity. Recently, the gut microbiota demonstrated to modulate the gut-brain signalling in physiopathology in what has been called microbiota-gut-brain axis (MGBA) [1]. Gut microbial dysbiosis increases local inflammation also thanks to the contribution of bacterial lipopolysaccharides (LPS), which disturbs the gastrointestinal and also the blood-brain barrier (BBB) permeability thus possibly propagating inflammation to the brain where it may trigger acute or chronic neuronal damage. Consequently, elucidating MGBA impairment is a key challenge that may open to therapeutic approaches in many neurological diseases. In this perspective, body-on-chips seek to offer a tool in clarifying inter-organ cross-talks by proposing engineered solutions towards meaningful interconnected models of organs functionality *in vitro* [2].

The gold standard for modelling *in vitro* the biological barriers of the MGBA are Transwell®-like inserts that are particularly suitable to study transport of molecules by means of permeable supports in a bi-compartmental well. However, they do not reproduce flow perfusion and 3D environment, which demonstrated to dictate barrier morphology, transport properties, and allow for multiorgan communication. To overcome these limitations, within the ERC project "MINERVA" we have developed and tested a novel MGBA multiorgan-on-a-plate engineered platform hosting a modular organ-on-a-chip (MINERVA 2.0) connected in series that is also suitable to be interfaced with conventional bioreactors for bacterial culture. The platform has a size comparable with

a standard multiwell plate and can be hosted on the microscope holder. It features five engineered cell based models to recapitulate the main biological systems involved in the MGBA: intestinal microbiota, gut, immune system, BBB and brain. Each device allows dynamic culture for the specific tissue/system and real-time monitoring of biological barriers functionality by optical accessibility and integrated trans-endothelial/epithelial electric resistance evaluation. We present the platform development and assessment demonstrating its suitability for modelling *in vitro* both physiological and pathological MGBA, by addressing LPS transport, and for potentially performing *in vitro* drug efficacy and toxicity tests.

References

[1] Cryan et al. (2019). *Physiol Rev*. doi:10.1152/physrev.00018.2018

[2] Ingber (2022). *Nat Rev*. doi:10.1038/s41576-022-00466-9

Presentation: Poster

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Respiratory syncytial virus infection of peripheral nerve and spinal cord immunocytes induces delayed transient peripheral nerve hyperreactivity and persistant spinal cord infection

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Respiratory syncytial virus (RSV) is a common cause of lower respiratory infections and was previously thought to have exclusive tropism for the airway epithelium. However, recent studies suggest that this virus can infect non-respiratory cells, such as immunocytes and neuronal cells. Yet, the relationship between immunocytes, neuronal cells, and the mechanisms of neuronal injury in the context of RSV infection remain unclear. We used innovative microphysiological culture of embryonic rat dorsal root ganglion (DRG) and spinal cord dorsal horn (SCDH) cells to compare peripheral and central nervous system (PNS and CNS) susceptibility to RSV infection and establish a microphysiological model of the resulting functional deficits. Exposure of microphysiological nerve cultures to RSV is not followed by replication within nerve cells, but rather leads to transient infection of local macrophage-like cells followed by persistent infection of dendritic cells. Importantly RSV replication within these non-neuronal cells adjacent to nerve axons is associated to delayed transient peripheral nerve hyperreactivity following electrical stimulation and persistent infection of spinal cord tissue. Our findings show that RSV can modify neuronal transmission via infection of non-neuronal, mononuclear immune cells within peripheral nerves, and provide a plausible mechanism for the persistent airway hyperreactivity and other neuronal sequelae of RSV infection. Additionally, spinal cord immunocytes may provide a reservoir for long-term survival of RSV in the nervous system.

Presentation: Poster

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Human microphysiological model of afferent nociceptive signaling

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Conventional drug development pipelines have failed to produce safe and effective alternatives to opioids increasing the demand for novel approaches to analgesic characterization. We have developed a human pluripotent stem cell (hPSC)-derived microphysiological nerve tissue system modeling pain signal relay from peripheral nociceptors to the spinal cord dorsal horn (SCDH). hPSC-peripheral and SCDH neurospheres mature directly on an integrated, 3D Microelectrode Array (MEA) enabling repeated selective stimulation and bioelectric recording of each neurosphere.

We first derived a highly neuronal human cervical hPSC-SCDH population. When co-cultured with commercial hPSC-peripheral nociceptor neurospheres, single cell RNA sequencing revealed the development of dorsal horn interneuron (dI) subtypes 1, 2, 3, 4/6, and 5 with dI4/6 and dI5 subtypes favoring late-born phenotypes of SCDH lamina 1 which are innervated by DRG cells *in vivo*. We are currently investigating whether extended co-culture will result in increased levels of NK1R expression, which binds the DRG-derived pain transmitter substance P.

To investigate biomimicry of the hPSC-SCDH-DRG MPS, we examined maturation of circuit-level neurophysiology and morphine sensitivity over time with standard extracellular field potential recording. Immature spinal cord spheroids showed no spontaneous activity, but a complex glutamatergic, GABAergic, and morphine-sensitive waveform can be evoked through electrical stimulation of the afferent, synaptically-connected nociceptor spheroid. More mature cocultures generate fully spontaneous complex waveforms which increase in frequency upon morphine application. Pharmacological studies indicate that morphine disinhibits concerted glutamatergic circuit firing by silencing the GABAergic circuits that limit their activity within the hPSC-SCDH spheroid.

Finally, this microphysiological system was integrated with polycarbonate/stainless-steel, 3D microelectrode arrays (MEAs) using novel polydopamine-mediated silicon dioxide-based functionalization and dielectric definition. Two different technologies, polymer-hypodermic needle and glass-stainless steel, have been prototyped with 5-6 3D microelectrodes and characterized for microfabrication, SEM, electrical and optical metrics, and biocompatibility of surface layer insulation. We confirmed that both synaptically-evoked and spontaneous complex waveforms can be recorded through integrated MEAs after six months in culture, though optimization and scaling of MEA integration remain in progress.

Fully characterized 3D MEA-integrated microphysiological systems will offer a viable human tissue-based, low-cost, and higher-throughput alternative to *in vivo* experimentation for characterization of emerging pain-modulating compounds.

Presentation: Poster

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Evaluation of the gut-protective aerobic *Lactobacillus rhamnosus* GG bacteria on the colon intestine-chip

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Background: Host-bacterial microbiome interactions play crucial roles in homeostasis, health, and disease. While microbiota dysbiosis can drive inflammatory pathologies, such as inflammatory bowel disease (IBD), therapeutic probiotic bacteria can restore homoeostasis. Physiologically relevant *in vitro* human models for assessing host-microbiome crosstalk remain highly limited. Emulate's S1 Colon Intestine-Chip provides a physiologic human organoid-based platform for studying the intestinal epithelium. *Lactobacillus rhamnosus* GG (LGG) is a widely used probiotic that suppresses inflammation and promotes epithelial barrier function *in vivo* through its metabolites. The goal of this study was to evaluate the ability of LGG to protect epithelial integrity against inflammation in the Emulate S1 Colon Intestine-Chip.

Methods: We seeded human colonic intestinal microvascular endothelial cells in the bottom channel and colon organoid-derived fragments in the top channel of our S1 Chip and allowed them co-differentiate for the next 4 days. On day 5, a subset of Chips was pretreated (primed) with LGG-conditioned media for 24 hours, followed by addition of LGG bacteria. Simultaneously, a subset of Chips was treated with IFNγ, as an inflammatory barrier challenge, -/+ co-administration of the barrier protective JAK inhibitor, Tofacitinib. Epithelial responses were tracked from day 4 to day 8 via imaging, fluorescent tracer analysis of permeability, and MSD measurement of inflammatory cytokine release (e.g., IL-1β, IL-6, IL-8).

Results: Brightfield/fluorescent imaging and permeability measurements revealed the generation of a morphologically and functionally mature epithelial barrier by day 4 of the study. As expected, after 72 h IFNγ treatment, we observed clear evidence of colonic epithelial injury including loss of both cell border definition and ZO-1 staining, as well as increased permeability and elevated inflammatory cytokine release. All of these responses were effectively prevented by Tofacitinib. Remarkably, in Chips pre-treated with LGG we observed similar levels of epithelial barrier protection and suppression of inflammation.

Conclusion: These studies indicate that Emulate's S1 Colon Intestine-Chip is capable of recapitulating established *in vivo* therapeutic effects of both a pharmacologic and an LGG probiotic therapeutics. Moreover, this model may have broader utility in evaluating probiotics and other IBD therapeutic strategies.

Presentation: Oral

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Development of cell culture environment sustaining MPS (CCES-MPS) integrated with a dialysis membrane

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Microphysiological system (MPS) is attracting attention as a novel evaluation system for pharmacokinetics in drug discovery. However, conventional MPS cannot fully reproduce the drug concentration changes *in vivo* due to the necessity of culture medium change. The drug concentration in the blood administered *in vivo* changes continuously by absorption, distribution, metabolism, and excretion (ADME). In contrast, the drug concentration in MPS is intermittent because the concentration of drugs and metabolites in the culture medium cannot be maintained during medium change. A cell culture environment that continuously changes drug concentrations as *in vivo* is necessary to accurately predict and evaluate pharmacokinetics using MPSs. We have developed a Cell Culture Environment Sustaining MPS (CCES-MPS), based on Kinetic-pump integrated Microfluidic Plate [1], toward reproducing continuous changes in drug concentrations.

The CCES-MPS consists of two cell culture parts by connecting with microchannels, a dialysis part, and a stirrer-based micropump. Cell culture and medium sampling are easy because the cell culture parts are open chambers and the same shape as 24-well plate wells. The dialysis part is divided by a dialysis membrane into an upper layer that is a reservoir of culture medium to nutrient supply and a lower layer that is connected to the microchannel. The dialysis membrane has a mass selectivity that does not allow substances with a molecular weight higher than the Molecular Weight Cut-Off

(MWCO) to permeate. Therefore, drugs with molecular weights higher than the MWCO remain in the lower layer, while nutrients with smaller molecular weights diffuse from the upper layer to the lower layer. Thus, allow nutrients to be supplied to cells while keeping the drug concentration in the lower layer by changing the medium only in the upper layer. We confirmed that cell culture is possible using only nutrient supply through the dialysis membrane.

The CCES-MPS is expected to be a useful assay tool for drug discovery because it can reproduce drug concentration changes similar to *in vivo*. In this presentation, we introduce the overview of the CCES-MPS and the drug efficacy test using the CCES-MPS.

Reference

[1] Shinha et al. (2021). *Micromachines*.

Presentation: Poster

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Development of kinetic-pump integrated microfluidic plate (KIM-plate) for commercialization

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Microphysiological systems (MPSs) including organ-on-a-chip (OoC) have attracted attention as a novel method for estimating the effects and side effects of drugs in drug discovery. Conventional MPSs were connected to pump systems to perfuse culture medium to reproduce the dynamic *in vivo* environment. Therefore, most MPSs are not user-friendly and have low throughput. We developed a kinetic-pump integrated microfluidic plate (KIM-Plate) by applying the stirrer-based micropump to an open-access culture plate to improve the usability of MPSs. We aim to commercialize the KIM-Plate as a project of the Japan Agency for Medical Research and Development (AMED) in collaboration with Japanese manufacturing companies.

The KIM-Plate has a simple structure consisting of open-type 24-well size cell culture chambers connected by microchannels. The greatest advantage of the KIM-Plate is that users can perform perfusion culture and coculture with the same feeling as conventional culture methods. In addition, differentiation and functional evaluation can be performed for each organ model by using commercially available culture inserts and cell desks. Therefore, the effects of coculture and perfusion can be evaluated in detail using highly conditioned organ models. The kinetic pump can perfuse medium by rotating the stirrer bar with a magnetic stirrer motor. Therefore, the KIM-Plate is a very simple operation for perfusion, as it only needs to be installed on a stirrer base with a stirrer motor.

The KIM-Plate is useful as an evaluation system for organ interaction from the results of coculture experiments of the gut and liver models (Shinha et al., 2021). Currently, we are conducting drug evaluation tests using chimeric mouse-derived human hepatocytes and cancer cells to demonstrate the usefulness of the KIM-Plate as a novel drug assay system. The results of the experiments show the usefulness of perfusion culture and coculture using the KIM-Plate as an evaluation system for drug efficacy and toxicity.

The KIM-Plate is expected to facilitate high-quality cell-based assays in drug discovery and biology credited to its ease of use and high throughput. In this presentation, we present an overview of the KIM-Plate and details of the drug efficacy study.

Presentation: Poster

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Leveraging population of model *in silico* approach for robust islet tissue development in microphysiological systems

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Stem cell-derived β cells offer an alternative to primary islet cells in generating MPS tissue models. Still, issues of functional immaturity and an evolving concept of characterization of islet heterogeneity provide challenges in designing *in vitro* tissues that mirror the *in vivo* behavior. This has recently been shown in our islet β MPS, where hPSC-derived β cells often displayed glycolytic bottleneck [1]. Intercellular heterogeneity in islets results from variation in non-heritable responses to the microenvironment (i.e., phenotypic variation) or heritable responses resulting from evolutionary processes (clonal or genetic) resulting in β-cell subpopulations expressing varying levels of secretory roles. In MPS islet technology, the current approach to negotiating this challenge has been to explore heterogeneity via picking clones with improved glucose responsiveness through an array of monoclonal antibodies against cell surface markers, missing out phenotypes that may not be responsive yet important in the overall functional coupling in the 3D islet tissue. In this study, we present a population of model (PoM) approach to reconstruct inter-cellular heterogeneity *in-silico*, i.e., collections of computational cells created via Monte Carlo variation of model parameters. Since the robust secretion of insulin by the β-cell upon glucose challenge relies heavily on the coupling of the metabolic oscillations in the glycolysis pathway with electrical oscillations (i.e., action potential spiking and bursting), we defined parameter variation based on experimentally observed heterogeneity in ion current conductances and enzymatic affinities. With this

population of *in-silico* β-cells, we applied a set of experimentally defined criteria that captured physiologically relevant subclasses of β-cell and whole-islet function. We then performed a range of statistical approaches to relate those functional classifications to the underlying parameter variations, thus indicating how specific biochemical mechanisms are likely to influence the behavior of the heterogenous tissue. These procedures allowed us to discriminate how both glycolytic and electrophysiologic components of the β-cell machinery can render intact islets (primary or hiPSC-derived) incapable of glucose-stimulated-insulin-secretion. This novel approach provides proof-of-principle for applying detailed biophysical and biochemical models to partition the roles of phenotypic, clonal, and genetic heterogeneity in determining hiPSC-derived β-cell and islet function.

Reference

[1] Goswami et al. (2022). *Lab Chip*.

Presentation: Poster

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Durable engineered extracellular matrices with tunable biophysical and biochemical properties for long-term microfluidic culture

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Microphysiological systems (MPS) incorporate cells into microfluidic devices to provide an *in vitro* physiological microenvironment for disease modeling and drug testing applications. Often, these MPS require an extracellular matrix (ECM) to physically support the cells within the device. Beyond simple physical support, the ECM is a crucial determinant of cell behavior and function. The ECM provides extrinsic biophysical and biochemical cues that can influence cellular decisions such as proliferation, migration, and differentiation. As such, the ECM is a crucial regulator of *de novo* tissue formation *in vitro*. However, cells constantly remodel their microenvironment through degradation and redeposition of the ECM, making regulating these cues over time to guide tissue development challenging. Additionally, long-term cultures of MPS containing ECM-supported cells often fail due to cell-mediated degradation and compaction of the ECM. To address these challenges, we developed a modified collagen-based ECM compatible with microfluidic devices in which biophysical and biochemical properties can be easily and independently tuned to instruct cell behaviors within MPS and provide a durable ECM for long-term cultures. Specifically, we developed a chemically modified collagen-based ECM via a reaction of collagen I with Norbornene. The stiffness of this engineered ECM can then be tuned both by varying the collagen concentration and viscoelasticity through the addition and over time by diffusion of tetrazine (Tz)-containing crosslinkers that react in a highly selective inverse electron demand Diels-Alder reaction after the native collagen gelation. Additionally, the introduction of Tz-containing crosslinkers can modulate the viscoelastic properties of the ECM. Utilizing this system, we have generated ECM with shear moduli between ~200-800Pa with independent control over viscoelasticity, as indicated by differing stress-relaxation times under constant deformation of ~4 orders of magnitude for equivalent stiffness conditions. Lastly, interpenetrating networks of alternative ECM proteins can provide distinct biochemical cues to direct tissue development independently of mechanics, and the mechanical crosslinking be tuned to resist cell-mediated compaction within devices. Together, this material represents a unique engineering platform that allows for regulating ECM-mediated cues in MPS and long-term culture in microfluidic systems.

References

[1] Watt, F. M. (2013). doi:10.1038/nrm3620
[2] Hagiwara, M. (2021). doi:10.1038/s42003-021-02350-4
[3] Chaudhuri, O. (2020). doi:10.1038/s41586-020-2612-2

Presentation: Poster

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Heuristic method for the discovery of a common media to support integration of a hiPSC-derived type 2 diabetes mellitus microphysiological system

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Combining hiPSC-derived tissues with MPS platforms promises to model human diseases involving tissue crosstalk, such as interactions between adipose, hepatocytes, β cells, and macrophages in obesity-induced type 2 diabetes mellitus (T2DM). However, a major impediment to the integration of multiple tissue MPS is common media allowing metabolic recapitulation of the native tissue interactions. To solve this issue, we incorporated a differential evo-

lution algorithm to identify common medium circulating between liver, islet and adipose MPS [1,2]. First, we identified a commercially available RPMI-based human plasma-like media (HPLM) [3] as the core medium for the five cells/tissues. Second, we optimized factors/components required for the maintenance of each tissue including macrophage stimulating factor (MCSF), thyroid hormone T3, and hepatocyte growth factor (HGF). Media variants were created based on the control hyper-parameters of the algorithm such as mutation, crossover, and the existing state of the variant population. We observed that HPLM alone induced inflammation in the M0-macrophages as measured by a cytokine panel but improved the insulin responsiveness of adipocytes. We have identified two specific regions in the parametric space of the components that are associated with four optimal common media variants. We also discovered media variants whereby the components added to HPLM allowed macrophages to maintain phenotypic stability, and the islet β cells to have a higher glucose-stimulated-insulin-secretion compared to a popular commercial islet medium. Similarly, adipocytes demonstrated better insulin response (via glucose and fatty acid uptake) versus base medium alone, while hepatocytes maintained albumin secretion at the same level as commercial hepatocyte medium. Optimized media will be tested in our liver-islet-adipose MPS to measure the functionality of tissues before and after native tissue interaction, paving the way for the development of an MPS platform for formulating better preventive, diagnostic, and treatment strategies for obesity-induced T2DM.

References

[1] Goswami et al. (2022). *Lab Chip 22*, 4430-4442.
[2] Loskill et al. (2017). *Lab Chip 17*, 1645-1654.
[3] Cantor et al. (2017). *Cell 169*, 258-272.

Presentation: Oral

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Embracing complexity to increase efficiency and predictivity: High-throughput 3D microfluidic modeling of drug-induced liver injury powered by image-based AI toxicity profiling

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Traditional drug discovery is a long and costly process, with a high failure rate. While drugs undergo rigorous preclinical pharmacology and toxicity assessment, many drugs fail during clinical trials due to subsequent hepatotoxicity detected in patients, with drug-induced liver injury (DILI) remaining one of the most common causes of safety-related drug marketing withdrawals. This toxicity discrepancy is due to a lack of drug safety predictivity in traditional animal models. Towards this, we have developed a DILI model using our validated liver chip platform to perform high-throughput drug toxicity testing. Our proprietary microfluidic device combines microstructure patterning and surface functionalization to achieve membrane-free compartmentalization of multiple microfluidic channels that are amenable for biomimicry, scalability, and optical compatibility for high-resolution microscopy. The workflow for in-house hepatotoxicity assessment relies heavily on the automation compatibility of our device, incorporating lab robotics to increase efficiency and throughput. Fully automated chip seeding results in efficient and reproducible extended co-culture of primary human hepatocytes and liver sinusoidal endothelial cells on our device, allowing establishment of tissue architecture and functional maturation. Mature liver chips undergo acute compound exposure at clinically relevant doses via perfusion. During this exposure, tissues are assessed for hepatotoxicity-related biomarkers via kinetic sampling. At endpoint, tissues are subjected to CellPainting, followed by fully automated HT/HC imaging and AI-guided image analysis. Integrating the multiplexed biomarker data with endpoint image-based phenotypic data demonstrates highly sensitive detection of hepatotoxicity. Our findings support further development of combinatorial approaches to DILI, combining human liver chip models with image-based profiling for next generation predictive toxicology.

Presentation: Oral

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Application of renal proximal tubule-on-a-chip: Challenge and benefit for supporting drug development in a pharmaceutical industry

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Clinical adverse events are serious concerns during drug development and are even observed for marketed medications. Pharmaceutical industries participating in the Innovation & Quality Microphysiological Systems have documented contexts of use (COU) for complex *in vitro* models (CIVM) to reduce drug attrition within the drug development process by integrating CIVM technologies [1]. CIVM are currently recognized as better tools to model physiologically relevant human organs and, thus, may potentially assist to predict drug-induced toxicity issues. Indeed, nephrotoxicity represents a major liability in certain drug classes. Nephrotoxicants primarily target specific regions of the nephrons – glomeruli and

proximal tubules (PTs) – to induce renal injury during drug clearance, reabsorption and accumulation processes. A number of compounds have been experienced dose-limiting nephrotoxicity and caused renal injury in preclinical studies. Accordingly, there have been urgent needs of adequate *in vitro* models to overcome renal safety concerns. Here, we share our evaluation of a 3-dimensional (3D) PT model in a microfluidic device for COU, and our case studies for supporting our portfolio. Both human and rat PT cells were used for interspecies comparison of nephrotoxicity. Proof-of-concept studies using various experimental parameters – mitochondrial function, necrosis, lactate dehydrogenase production, kidney injury markers and inflammatory markers – demonstrated that 3D human PTs exposed with known nephrotoxicants such as polymyxin B exhibited reproducible outcomes of nephrotoxicity as previously reported. A rat 3D PT model recapitulated bisphosphonate-induced nephrotoxicity as previously seen in *in vivo* studies. Evaluation of internal portfolio compounds led to differentiated safety profiles based on species and parameters such as PT damage, cytotoxicity, oxidative stresses, and injury/inflammatory biomarker production. Furthermore, the test compounds caused renal injury in the rat PTs as observed in rodent studies. These findings suggest potential translatability between *in vitro* and *in vivo* settings, improved identification of renal safety concerns and nephrotoxicity prediction for candidate compounds during drug development. Overall, this presentation will highlight our experience in the use of 3D PTs over the past few years, including challenges and benefits, for drug safety assessment in the pharmaceutical industry.

Reference

[1] Baran, S. W., Brown, P. C., Baudy, A. R. et al. (2022). Perspectives on the evaluation and adoption of complex in vitro models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate). *ALTEX 39*, 297–314. doi:10.14573/altex.2112203

Presentation: Oral

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Development of a culture medium perfusion platform improving usability of microphysiological systems

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Recently, microphysiological systems (MPS) have attracted attention as a novel *in vitro* cell-based assay system for pharmacokinetics, pharmacodynamics, and toxicity assessment as an alternative method to animal experiments in drug development (Zilberman et al., 2021).

As organ models such as BBB, kidney, and gut MPSs using two-compartment microfluidic chips separated by a porous membrane have been widely proposed (Kimura et al., 2017). Many of these MPSs have perfusion systems that mimic *in vivo* flow to maintain the original organ function and morphology of cultured cells to evaluate cells in conditions similar to the physiological environment. However, these perfusion systems have complex setups for pumping such as numerous pumps and long tubes, making system operation difficult.

Therefore, we have developed a user-friendly culture medium perfusion platform that integrates pumps, culture medium chambers, tubes, and two-compartment microfluidic chips into an ANSI/SLAS standard-sized plate, which can be used for microscopic observation and evaluation using a measuring instrument under perfusion culture.

The culture medium perfusion platform was made of biologically synthetic materials with low cytotoxicity using a 3D printer. Independent culture medium chambers allow for medium perfusion for each upper and lower channel on the two-comportment microfluidic chip. Also, various levels of shear stress loading can be applied by adjusting the voltage to control the flow rate of the pump. The culture medium perfusion platform and the two-compartment microfluidic chips are held in place by magnets, allowing for easy attachment and removal. The platform enables easy cell culture and evaluation of cell morphology and function under shear stress loading conditions using MPSs.

In this presentation, we will present an overview of the culture medium perfusion platform and details of cell culture tests using the platform.

Presentation: Poster

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Customizable gut-on-a-chip microsystems with enzymatic digestion for food and drug studies

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With our ability to integrate an ever-wider range of physiologically inspired functions into an organ-on-a-chip, we can customize devices to be increasingly fit for purpose. For instance, intestine-on-chip (or gut-on-chip) devices have to date focused on mimicking the *in vivo* intercellular interactions at the intestinal wall that control the absorption of compounds over this biological interface.

However, the compound of interest arrives at the intestine after having been ingested as a medicine containing excipients or as a food matrix. Enzymatic digestion serves to release and change the form of the compound as it passes from the mouth through the stomach to the intestine. Therefore, to study the uptake of novel drugs, toxicants, or food materials, a fit for purpose organ-chip should include an *in vitro* digestive system in addition to intestinal absorption, to provide valid information on bioavailability (the fraction of compound that finally reaches the bloodstream).

The work described here considers on-chip digestion, a process requiring multiple functions, for eventual integration with a gut-on-a-chip. After earlier work that mimicked adult digestive processes [1], we now present a versatile system to emulate the digestion of infants. We translated a static, batch-wise, *in vitro* digestive system for *à terme* infants [2] into a continuously flowing, infantile, digestive system. Sequentially linked microfluidic micromixers were employed as microreactors to perform specific digestive processes. Artificial digestive juices were prepared at defined compositions and pH, to ensure exact physiological conditions at each stage (microreactor) of infantile digestion. All of the flows in the on-chip digestive system and the gut-on-a-chip were maintained by a novel flow control system, based on Coriolis mass flow sensors to maintain exact flow rates irrespective of digestive-juice properties [3]. Operation of the organ-chip was possible for a period of 13 days with no replacement of medium reservoirs required. We demonstrate the digestion of lactoferrin benchmarked against currently used *in vitro* digestion models.

References

[1] de Haan et al. (2019). *Lab Chip*. doi:10.1039/C8LC01080C
[2] Ménard et al. (2018). *Food Chem*. doi:10.1016/j.foodchem.2017.07.145
[3] Sparreboom et al. (2013). *Micromachines*. doi:10.3390/mi4010022

Presentation: Poster

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A human iPSC-based *in vitro* neural network formation assay to investigate neurodevelopmental toxicity of pesticides

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The spatiotemporal orchestration of key neurodevelopmental processes (KNDP), including the formation and function of neural networks (NNF) is essential for proper brain development. An adverse outcome, i.e., developmental neurotoxicity (DNT), is expected, if at least one KNDP is affected due to exposure towards a compound during a critical period of neurodevelopment. To allow a higher testing throughput than the guideline animal experiments (OECD TG426), a developmental neurotoxicity (DNT) *in vitro* testing battery (DNT-IVB) has been set up, which models several KNDPs based on different cell models. Gap analyses of the DNT-IVB revealed the need of a human-based assay to assess NNF. Therefore, here we established the human NNF assay. A co-culture comprised of human induced pluripotent stem cell (hiPSC)-derived glutamatergic excitatory and GABAergic inhibitory neurons, as well as primary human astroglia, was seeded in a defined cell type ratio and differentiated for 35 days on 48-well micro-electrode arrays (MEA). The spontaneous electrical activity, together with cytotoxicity, was assessed on a weekly basis after washout of the compounds 24 hours prior to the measurements. In addition to the characterization of the test system, the assay was challenged with 28 compounds, mainly pesticides, identifying their DNT potential by evaluation of specific spike-, burst- and network- parameters. This approach confirmed the suitability of the assay for screening environmental chemicals. Together with the successful implementation of hNNF data into a postulated adverse outcome pathway (AOP) network on deltamethrin exposure, we suggest the hNNF assay as a useful complement to the current DNT-IVB.

Presentation: Poster

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Systemic & high-throughput: Liquid microphysiological systems

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In vitro characterization of drugs and chemical compounds is essential to predict their effects on patients and consumers. Unfortunately, the results obtained with current *in vitro* assays are poorly reliable because they do not faithfully recapitulate the organization of the human body. An important missing piece in these assays is the ability to mimic the interactions between human organs. Solutions like the Body-on-a-chip were developed to mimic the systemic organization of the human body. However, these microphysiological systems are poorly flexible to adapt to diversified projects. Moreover, they are not compatible with high-throughput screening to address the need to test thousands of molecules with the predictive power of such systemic assay. To solve this technological limitation, we have invented a patent-pending method (WO2021058557A1) to generate liquid microphysiological systems.

The technology of FluoSphera allows the design of multi-tissue systems in multi-well plates. Multiple human tissues are growing in 3D (spheroids or organoids) within fluorescent capsules (one color for each tissue type). Encapsulated tissues are then mixed into a culture well to create a human multi-tissue system (biosystem). In such biosystems, the effects of a drug/compound are similar in many aspects to what is truly observed in patients since all the tissues can interact together. For the acquisition of experimental data, we use High-Content Screening (HCS). HCS can individually identify each tissue of our biosystem thanks to the color-coded capsules. After the addition of specific fluorescent reagents or by using fluorescent reporter genes, biological effects can be specifically measured in each tissue type (readouts). Our technology thus allows the quantification of the systemic effects of a compound, thus generating data that are much more predictive than current *in vitro* testing.

Our method can address questions on multi-tissue systemic effects of compounds like the effects of endocrine disruptors, but also the effects of liver drug metabolism on other tissues (indirect toxicity, activation/inactivation of drugs, etc.). Currently, we are developing an endocrine system to characterize endocrine-disrupting chemicals and drugs, and a liver-heart system to better detect drugs and compounds showing direct or indirect cardiotoxic effects.

Presentation: Poster

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Soldier-on-a-chip: Interrogating the effects of chemical and biological threat agent exposure using a multi-organ microphysiological system

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Predicting the toxicity of various chemical and biological agents of concern has traditionally relied on research using two-dimensional cell lines in culture, or *in vivo* models. However, cell lines aren't always physiologically accurate, and *in vivo* experiments can be costly and time consuming. Organ-on-a-chip technologies, otherwise known as microphysiological systems (MPS) have been gaining popularity as models that are more predictive measures of human outcomes for biological and chemical exposures. While MPS have emerged as a viable alternative to relying solely on complex animal studies, a limitation to the vast majority of commercially available systems is the inability to run multiple organs or organ systems in one chip. The chips designed and fabricated by TissUse have overcome this limitation. The TissUse multi-organ-chip (MOC) is the size of a microscope slide and uses micro-pump enabling pulsatile flow. It is amendable to iPSC-derived cells, primary cells, organoids, or cells cultured in trans-wells. By integrating multiple target organ systems on one chip, the TissUse system potentially allows for a more complete and physiologically relevant picture of the effects of chemical and biological agents of concern and provides the user the ability to identify both primary and secondary target toxicity. Moreover, the ability to collect 'omics data from multiple human organ systems from a single chip saves time and money, by increasing throughput, and organ system coverage. Here, we have created a soldier-on-a-chip toxicity analysis pipeline by combining the TissUse MOC technology with single chip multi-omics analysis on a global scale. We have integrated heart, lung, and skin on to one chip to recreate complex biological functions, which allows for a more comprehensive analysis of the physiological response to agents of concern as compared to single-organ-chip systems. In this study, chips were exposed to chemical or biological threat agents directly onto the lung tissue, which resulted in dysregulations across a wide array of cellular processes in all three organ systems including cell structure, transcription, lung signaling, apoptosis, cardiac calcium flux, inflammation, and angiogenic suppression.

Presentation: Poster

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Towards a microfluidic approach for assessing effects of spatiotemporal oxygen fluctuations in the tumour microenvironment

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Within solid tumours, tumour cells display uncontrollable proliferation. Simultaneously, the surrounding vasculature is often inadequately formed and as such, incapable of fully supporting the metabolic needs of the tumour cells. Together, this leads to the formation of spatial and temporal fluctuations in oxygen tension, resulting in intermittent and continuous hypoxia inside the tumour microenvironment (TME) [1]. The consequently heterogeneous microenvironment affects tumour cell metabolism, proliferation, migration and invasion and their response to chemotherapeutic agents [2]. However, unravelling the mechanisms governing cellular behaviour in spatial and temporal oxygen gradients in cancer biology is limited by the lack of appropriate biological model systems [3]. Here, we describe a bioengineered 3D microfluidic cancer-on-chip system, which allows the analysis of tumour cell behaviour in oxygen environments that can be spatially and temporally varied. The chip was established by micromilling of polymethylacrylate (PMMA) and consisted of a round shaped cell culture chamber, surrounded by a supply channel for cell culture medium. By exploiting the microfluidic chip design and flow rate, a uniform radial oxygen gradient from 21% oxygen down to 0.3% toward the centre of the cellular compartment can be achieved (further verified computationally using COMSOL). For proof of principle, we cultured MDA-MB-231 breast cancer cells within a collagen-I matrix in the chip. The oxygen gradient was validated using the hypoxia marker pimonidazole, showing an increase in signal in the cells towards the core of the microfluidic device. Temporal fluctuations in oxygen tension could be created by changing the flow rate for a certain amount of time during the culture of the cells. We are currently employing this device to explore the role of spatiotemporal fluctuations in oxygen tension on tumour cell proliferation, migration and metabolic activity, as well as drug efficacy.

References

[1] McKeown, S. R. (2014). Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. *Br J Radiol 87*, 20130676.

[2] Dewhirst, M. W. (2009). Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress. *Radiat Res 172*, 653-665.

[3] Ahmed, M. A. M. and Nagelkerke, A. (2021). Current developments in modelling the tumour microenvironment *in vitro*: Incorporation of biochemical and physical gradients. *Organs Chip 3*.

Presentation: Poster

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3D microvascular inflammation model incorporating human genetic diversity amenable for microphysiological systems

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Exposure to pathogens (viruses, bacteria) and xenobiotics (heavy metals, micro-, and nanoparticles, agrochemicals) often leads to microvascular inflammation (MVI). MVI can be both a starting point and a major contributor to the progression of many disorders (e.g., obesity, diabetes, coronary microvascular disease, chronic kidney disease, inflammatory bowel disease, and immune rheumatic diseases) affecting large portions of the human population. Advancing research in this field may help to improve MVI detection and treatment.

Particularly, there is a need for an assessment that is specific to the pro- or anti-inflammatory activity of chemicals (drugs, toxicants) in the microvasculature. However, this research faces several obstacles. First, MVI in animals rarely correctly portrays MVI in humans, and animals cannot provide the throughput necessary for early discovery studies. Secondly, traditional cell-based models of MVI cannot represent blood capillaries, which 3D geometry strongly impacts the molecular physiology of endothelial cells (ECs) that make up the capillaries. In addition, *in vivo*, the flow of blood strongly affects their responsiveness to external factors (signaling molecules, drugs, or toxicants). Therefore, the preferable *in vitro* MVI model is a capillary organoid that can be perfused in a microphysiological system (MPS).

We have developed a 3D platform technology for making human capillaries *in vitro* that is amenable to use in MPS. We make the capillaries from human donor-specific endothelial progenitor cells (EPCs) – physiologically relevant vasculogenic cells normally present in human circulation. EPCs can be isolated from neonatal and adult blood and grown in large quantities using our proprietary EC growth media Vecstem™ enabling reproducible results. We have successfully isolated and cryopreserved EPCs from many donors representing both sexes of different ages and ethnicity. Initially, we differentiated the neonatal EPCs into microcapillaries and demonstrated that the key proinflammatory cytokine TNF-α upregulated in EPCs the leukocyte adhesion molecule VCAM1,

which is a highly specific biomarker of MEI. We propose to use *in vitro* VCAM1 expression under conditions of flow in MPS to quantitate the pro- or anti-inflammatory activity of chemicals in genetically diverse human populations.

Presentation: Poster

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Monitoring of immune cell cross-talks and microdroplet/ aerosol transmission in lung microphysiological system

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Pioneers in organ-on-a-chip research have developed multiple alveoli-on-a-chip systems [1,2], but the mimic of interactions among immune cells and the virus spreading between chips are still missing [3]. Here, we report the development of a human lung physiological system (Lung-MPS) that allowed the involvement of multiple types of immune cells and had the setup that could produce microdroplet/aerosol transmit among chips.

The chip body was fabricated with polycarbonate and bio-compatible materials. Bronchi epithelial cells and type II pneumocyte-like epithelial cells, macrophages, together with vascular endothelial cells were loaded to different layers in the chip to form corresponding cell sheets. After one week cultivation followed with air-liquid stimulation, both the epithelial and endothelial tissue in the chips formed layers and maintained high confluence and viability. The system was activated with LPS, Covid-19 spike protein, pseudo-virus, and perfused with blood mimicking medium that contains immune cells to monitor inflammatory responses in the chips. Images was taken with self-developed high-content imaging system, and deep-learning program was also written by us [4].

After initial LPS treatment, macrophage activation, monocytes adhesion/aggregation together with decreases in the TEER values could be observed, indicating that the involvement of residential macrophages and circulating monocytes significantly aggravated inflammation. The expression of inflammatory cytokines, like TNF-α, IL6 and MCP1, significantly increased in MPSs with circulating monocytes compared with systems without them. An integrated microdroplet/aerosol transmission system was also fabricated to study the propagation of pseudo-virus particles among chips. Lastly, a deep-learning algorithm was developed to characterize the status of cells in Lung-MPS and achieved > 95% accuracy.

In conclusion, we fabricated Lung-MPS that allow immune cell cross-talks and microdroplet/aerosol transmission among them. This system could provide a sensitive and real-time tool for the study of COVID-19 and other high-risk pneumophilic infectious diseases *in vitro*.

References

[1] Low, L. et al. (2021). *Nat Rev Drug Discov 20*, 345-361.
[2] Huh, D. et al. (2000). *Science 328*, 1662-1668.
[3] Chen, Z. et al. (2023). *Biosens Bioelectron 219*,114772.
[4] Chen, Z. et al. (2021). *Biomaterials* 272, 120770.

Presentation: Oral

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Development of automated microphysiological systems and AI-based algorithms for drug evaluation

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Microphysiological system (MPS), or Organs-on-a-Chip system (OOC), is a new type of *in-vitro* biomedical device/system that aims to recapitulate organ-level tissue structures and functions. Its applications include drug-screening, disease modeling, precision medicine, and toxicology usage, etc. [1,2].

In the past ten years, we have developed multiple organs-on-a-chip systems including biomimetic heart, skin, blood vessel, kidney, liver, etc. [3-5]. Our previous work demonstrated that with microfabrication, microfluidics, advanced 3D printing [6], and tissue engineering techniques, the miniature tissue constructs could form tissue-specific structures and may maintain desirable organ functions for up to four weeks or more.

Here, we overview a few OOC systems that have been developed in our lab aimed for drug screening. We will demonstrate how the heart-on-a-chip could accurately predict drug efficacy *in vitro* – a new drug is currently in IND application, and how our skin-on-a-chip could monitor materials' toxicity with OECD standard. In addition, we will report how our tumor-on-a-chip model is able to assess cancer cell viability, migration, and metastasis characteristics in real-time effectively. The technologies we developed in past ten-years offer packaged-solutions to solve the problems in current MPS development, including: i) large-scale fabrication of tissue constructs [4], ii) precise while large-scale construction of ultra-fine 3D-microenviroments and 3D-structures with two-photon printing at a resolution around 150 nm [6], and iii) imaging of MPS with an automated system, and analyzing chip and organoids with deep-learning based AI-algorisms [5].

As a conclusion, our research provided new models, technologies, and AI-software for high-throughput drug-screenings with

MPSs. In all, working together with members in international MPS society, we will promote the MPS based evaluation of drug efficacy and toxicity tests becoming more and more human-mimetic, automatic, and intelligence.

References
[1] Low, L. et al. (2021). *Nat Rev Drug Discov 20*, 345-361.
[2] Zheng, F. et al. (2016). *Small 12*, 2253-2282
[3] Chen, Z. et al. (2023). *Biosens Bioelectron 219*,114772.
[4] Zhang, J. et al. (2021). *Lab Chip 21*, 3804-3818.
[5] Chen, Z. et al. (2021). *Biomaterials 272*, 120770.
[6] Liu, K. et al. (2022). *Nat Commun 13*, 4563.

Presentation: Poster

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Application of adipose-derived mesenchymal stem cell-derived small extracellular vesicles for bladder reconstruction

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Adipose-derived mesenchymal stem cell-derived small extracellular vesicles (Ad-MSC-sEVs/AMEs) combined with scaffold materials are used in tissue-engineered bladders; however, the lack of retention leads to limited distribution of AMEs in scaffold areas and low bioavailability of AMEs after bladder reconstruction. To improve retention of AMEs, we developed a novel strategy that modifies the surface charge of the bladder acellular matrix (BAM) via oxidative self-polymerization of dopamine-reducing graphene oxide (GO) and AMEs by ε-polylysine-polyethylene-distearyl phosphatidylethanolamine (PPD). We evaluated two BAM surface modification methods and evaluated the biocompatibility of materials and PPD and electrostatic adherence effects between PPD-modified AMEs and rGO-PDA/BAM *in vivo* and *in vitro*. Surface modification increased retention of AMEs, enhanced regeneration of bladder structures, and increased electrical conductivity of rGO-PDA/BAM, improving bladder function recovery.

Presentation: Poster

725

Ontologies as tools to support MPS-based predictive toxicity screening

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Ontologies are gaining momentum in the field of toxicology and risk assessment. Ontologies are defined as mode-of-action frameworks qualitatively and quantitatively integrating and structuring relevant biological, toxicological, chemical, and kinetic data from various sources. Ontologies have their roots in adverse outcome pathway networks, which in turn originate from physiological maps. Among other applications, ontologies can serve as the conceptual basis for setting up animal-free and human-relevant batteries for the toxicity testing of chemicals. This will be demonstrated in this presentation. Focus will be put on the liver, which is a frequent target for systemic toxicity because of its unique location and function in the organism. A tiered ontology-driven approach for the prediction of steatotic and cholestatic liver toxicity induced by chemicals and relying in part on MPS-based *in vitro* testing as well as on expression and functional analyses will be presented.

Presentation: Invited

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3D bioelectronic models of the gut, brain and lung

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Physiologically relevant *in vitro* human models are urgently required to bridge the gap between over-simplified 2D *in vitro* models and ill-suited animal models, for drug discovery and disease modelling. Bringing together principles of materials science, tissue engineering, 3D cell biology and bioelectronics, we are building advanced models of the gut, brain (neurovascular unit) and the lung. In the case of the gut and NVU, our aim is to elucidate the role of microbiota in the gut-brain axis communication, a particularly challenging system to model with lab animals. In the case of the lung, our interest is in modelling the air-liquid interface to more accurately study lung disease.

Our models are based on the use of electroactive scaffolds which can host tissues, but also monitor their formation, and later their status when challenged with a pathogen or target molecule. Our strategy is to build a stromal layer with the scaffold and layer epithelial or endothelial models on top, preserving tissue stratification. Our most recent work has adapted these scaffolds to the well-known Transwell format, to allowed continued access to both

apical and basal aspects.[1] As well as traditional readouts such as immunofluorescence, or biochemical analyses of media, our continuous electrical monitoring readouts from our electroactive scaffolds gives us real-time data on the tissue health.

Reference

[1] Pitsalidis, C. et al. (2022). Organic electronic transmembrane device for hosting and monitoring 3D cell cultures. *Sci Adv 8*, eabo4761. doi:10.1126/sciadv.abo4761

Presentation: Invited

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Oxygen-sensitive 3D cell culture systems – a tool for 3D mito stress tests

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Since 1953, about 500 drugs have been withdrawn from the market due to late-stage toxicity and unexpected side effects. This is mainly due to discrepancies in the results of *in vitro*-screening methods and human clinical trials. Current *in vitro*-methods are often based on monolayers, typically cultured under ambient atmosphere (around 18 to 21% O_2) although tissue O_2 levels are much lower (0.5 to 14%) which thus leads to hyperoxia, with the associated changes in the cells' metabolism leading to stress and a result bias in substance testing. In contrast, human 3D cell culture systems that generate O_2 gradients resembling those of *in vivo*-tissues provide much more relevant results. However, it is challenging to determine and adjust the O_2 concentration in 3D cultures in real time because most O_2 measurement techniques are not suitable for use in 3D cell cultures.

For the generation and cultivation of 3D cell aggregates, so-called polymer film-based microcavities were established at the Karlsruhe Institute of Technology (KIT). In a cooperation with PreSens Precision Sensing GmbH (Regensburg) we refined them in a way that they could be coated with an oxygen-sensitive fluorophore and allow the measurement of oxygen in the microenvironment of the cells by dynamic fluorescence quenching.

By using this sensor arrays, hundreds of data points per cm² are feasible, which makes the system useful for high-throughput screenings. Furthermore, in principle, it is possible to perform high-resolution confocal microscopy in the system in addition to oxygen measurements.

With first cellular models we were able to show changes in oxygen concentration during formation of spheroids and to measure the influence of respiratory chain inhibitors. In addition, a 3D mito stress test to characterize cellular respiration in mitochondria in more detail was established [1].

With the developed system, it is now possible for the first time worldwide, to determine the oxygen concentration and O_2-gradients in the immediate microenvironment of three-dimensional cell aggregates as well as in the surrounding medium.

Reference

[1] Grün, C. et al. (2023). O2-sensitive microcavity arrays: A new platform for oxygen measurements in 3D cell cultures. *Front Bioeng Biotechnol 11*, 1111316.

Presentation: Poster

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In vitro 3D human gingival tissue model to study oral microbiome interactions

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The oral cavity contains different microenvironments, as the non-shedding surface of the teeth and the epithelial mucosa, where oral barriers and microbial communities coexist. The interactions and balances between these two communities are responsible for oral tissue homeostasis or dysbiosis, that ultimately dictate health or disease. Disruption of this equilibrium is the first necessary step towards chronic inflammation and permanent tissue damage in the case of chronic periodontitis. There are currently no experimental models able to mimic the structural, physical, and metabolic conditions present in the oral gingival tissue to support the long-term investigation of host-pathogens unbalances. Herein, we report a 3D anatomical gingival *in vitro* model based on human primary culture that recapitulates the native tissue organization, and a native oxygen gradient within the gingival pocket to support human microbiome persistence with a physiologically relevant level of microbial diversity as well as native spatial organization. The modulation of inflammatory markers in the presence of oral microbiome suggested the humanized functional response of this model. The model will be used in future studies to investigate host-pathogen unbalances in gingivitis and periodontal disease.

Presentation: Poster

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Integrating human organoids into organismoids – how to achieve human body homeostasis *in vitro*?

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Over the last decade, a variety of microphysiological systems have been developed to emulate human biology at single and multi-organ level *in vitro* [1,2]. At the same time, the vision of achieving the ultimate organismal level on chips has advanced towards the organismoid theory [3]. Indeed, such (organismoid) miniature, mindless and emotion free, self-contained physiological equivalents of an individual patient's mature healthy and diseased body on a chip have the potential to revolutionise basic research into human biology, the development of curative therapies and the precision of personalised medicine. Stem cell technologies, organoid differentiation platforms and MPS-based artificial intelligence are converging to create the conditions for automated large-scale operation of such solutions in the future.

The keynote will highlight the main challenge in achieving body homeostasis on chips. This is our limited knowledge of nature's principles of genetically and microenvironmentally encoded self-organisation and maintenance of the smallest functional units of human organs and their integration into a human-like, efficiently interacting system of blood perfusion, innervation, immune surveillance and hormonal regulation of organs. It will discuss how we can take advantage for that ultimate step of what has been learned in recent years about the ability of human organoids to self-organise *in vitro*. The role of local segregation of the organotypic oxygen, nutrient and protein gradient-driven microenvironment of each organ equivalent from common blood flow, among others will be discussed. Finally, thoughts will be shared on how to make future MPS-based solutions affordable and sustainable on an industrial scale for the prosperity of the next generations.

References

[1] Marx et al. (2020). *ALTEX.* doi:10.14573/altex.2001241
[2] Roth et al. (2021). *Science.* doi:10.1126/science.abc3734
[3] Marx et al. (2021). *Front Medicine.* doi:10.3389/fmed.2021.728866

Presentation: Invited

730

Vascularised models for neurological disease

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Neurological diseases, including Alzheimer's and Parkinson's disease, affect approximately 50 million Americans each year, and with our aging population, this number will continue to grow. Despite two recently FDA-approved treatments, their success is limited, and new therapies are desperately needed. One increasingly valuable approach to address this need lies in the use of microphysiological models of the neurovascular system both to screen for new drugs and to test modes of delivery across the blood-brain barrier (BBB) into the brain. In this presentation I will describe the methods used for generating a microvascular network by natural self-assembly, and the morphological and functional characterization of the networks. The presentation will focus on development of an organ-specific network, the blood-brain barrier (BBB), in which a microvascular network is established in a microfluidic device that recapitulates human BBB function and morphology and can be combined with a neural compartment to capture the early stages of Alzheimer's disease. Experiments have been carried out for over 4 weeks either with or without continuous physiological perfusion. Examples will be provided focusing on the accumulation of amyloid beta at the vascular wall causing increases in vessel permeability and leading to neuronal dysfunction.

Presentation: Invited

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The female reproductive microphysiological system

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The first multi-organ microphysiological system of the female reproductive tract, EVATAR, was developed through a collaborative effort between biologists at Northwestern University and UIC, as well as engineers at Draper Labs. EVATAR was an integrated system that included the cycling ovary, fallopian tube, endometrium, ectocervix, and liver with continuous circulation of media within a microfluidic platform. As a result of this work, a second-generation LATTICE microfluidic platform was developed to provide a more affordable and reliable multi-well system that could be adopted by

researchers worldwide. LATTICE features a cell culture plate with eight wells that can house cells in monolayer or 3D cultures and are connected with microchannels. Physiologic culture mimics of polycystic ovarian syndrome (PCOS) including a hyperandrogenic ovary, fallopian tube, endometrium, adipose, and pancreatic islets were developed to study PCOS in LATTICE. Additionally, ovarian follicles were used screen over 52 environmental compounds for reprotoxicity. Based on years of collaborative work through the Tissue Chip Consortium, we have developed systems and models of the female reproductive tract that will lead to new discoveries and ultimately benefit women's health.

Presentation: Invited

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SMART organ-on-chip: From single chips to a standardized open technology platform

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Organ-on-Chip (OoC) is a game-changing approach in which human cells are cultured in microfluidic chips simulating and predicting the response of healthy and diseased human tissues. OoC has the potential to revolutionize today's biomedical testing procedures that often involve either conventional 2D cell testing with limited predictability, or ethically challenged animal testing which leads to variable results.

However, OoC research and development is still mainly happening in academic labs, where specific single chips are designed to answer specific questions, often with setups that are not user-friendly and that are not designed for scaled up production or experimentation. To take the next step and transfer organ-on-chip from academia to biological and pharmaceutical labs towards industrial adaptation, we are developing a standardized and modular open technology OoC platform, SMART OoC. This can accelerate OoC development by stimulating collaboration, and eventually enable the adoption of OoC by the biomedical and pharmaceutical industry. In this lecture, I will present the SMART OoC platform and sketch its current development and future possibilities.

Presentation: Invited

733

Functional bioengineered 3D neural models with neurovasculature to study neurological diseases and drug screening

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Three-dimensional neural tissue models are being developed to study brain development, neurological diseases and as physiologically relevant assays for drug discovery and development. Stem cell-derived neural organoids have been developed with complex neural cell composition and brain-like organization but lack vasculature and non-neural cell types like microglia. We are using bioengineering approaches to create functional neural tissue models that incorporate neurons, astrocytes, and neurovasculature. In one approach, we use hiPSC-derived neurons and astrocytes to form neural spheroids with designed neuronal type composition that mimics that of different human brain regions. We modeled two brain regions implicated in Parkinson's disease (PD) and Alzheimer's disease (AD), the ventral tegmental area (VTA) and prefrontal cortex (PFC). The "disease" neural spheroids for PD and AD were developed by incorporating genetically engineered neurons with mutations associated with high risk of developing each disease. These neural spheroids showed spontaneous network activity which was measured by intracellular calcium fluorescence imaging assay and was differentiated between healthy and disease models. In a second approach, we are using bioprinting technologies to create hydrogel-based engineered neural tissues with designed spatial layouts to create functional neural circuits and a neurovasculature unit tissue formed using primary brain endothelial cells, pericytes, astrocytes and neurons. Fluorescence biosensors are expressed in different neuronal types for real time measurement and optogenetics control of calcium oscillations and neurotransmitters release. Additional relevant cell types like microglia are now being included in these neural models to increase their physiological relevance to study neuroinflammation. The goal of these bioengineered neural assay platforms is to re-create complex functional neural circuits of the brain in a vascularized tissue to better mimic brain function and neurological diseases.

Presentation: Poster

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A patient-derived tumor organoid high-throughput screening platform for precision medicine

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Patient-derived tumor organoids (PDTOs) are representative of the native physiology of tumors across an array of malignancies, including sarcoma (Phan et al., 2019; Al Shihabi et al., 2021). We have established a fully automated, robust pipeline for facile, high-throughput organoid bioprinting and screening (Phan et al., 2019; Al Shihabi et al., 2021; Huyen et al., 2022; Tebon et al., 2023). Our platform is based on a modified geometry, with organoids grown as mini-rings or squares at the rim of wells (Huyen and Soragni, 2020; Tebon et al, 2023).

We have successfully generated organoids from over 200 samples thus far, originating from primary, recurrent, and metastatic rare cancers. In sarcoma, PDTOs closely resemble the tumor of origin in their histology and molecular features (Al Shihabi et al., 2023). Our scalable pipeline allows us to measure tumor-specific susceptibilities within a week from surgery and to recapitulate the observed clinical heterogeneity both across and within sarcoma subtypes. We identified patterns of response with respect to prior treatment, patient age, lesion type and disease trajectory (Al Shihabi et al., 2023).

Presentation: Invited

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Development of a highly differentiated human primary proximal tubule MPS model (aProximate MPS Flow™)

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The proximal tubule (PT) is the key nephron segment mediating renal drug elimination and is the primary site of drug induced nephrotoxicity. However, current animal studies have proved poorly predictive of human outcome. To address this there has been a recent upsurge in the production of relevant microphysiological systems (MPS) focused on replicating the PT functions *in vitro* and bridging the gap between *in vitro* and *in vivo*. Here we describe the preliminary results from our recently developed aProximate MPS Flow™ human PT platform, in which primary human PT cells are subject to fluidic media flow and a shear stress between 0.1-2 dynes/cm^2. Under fluidic flow conditions, human primary PT cells formed confluent monolayers with a Trans Epithelial Electrical Resistance (TEER) around 60-90 Ω·cm^2 by day 7 in culture, not significantly different from the 60-120 Ω·cm^2 of identical cells grown under static culture conditions. Similar results were obtained by measuring the barrier function using lucifer yellow (Papp 15.6 ± 06 cm/sec, n = 6). At the mRNA level for the key renal transporters, OAT1, OAT3, OCT2 MATE1, MDR1, megalin and cubulin, the expression levels were elevated at least 5-fold compared to identical cell monolayers grown under static conditions. Immunohistochemistry revealed that flow induced a significant increase in the number of primary cilia by cells within the monolayer with significantly higher expression levels of pericentrin and acetylated tubulin. Moreover, increased sensitivity to nephrotoxic protein cisplatin was seen, and creatinine and FITC-albumin uptake was significantly increased under flow conditions. Taken together, this dataset, suggests that growing human PT cells on Transwell filter supports with media flow across the apical membrane, significantly improves phenotype and function of PT cell monolayers and has significant benefit to the utility and near-physiology of the model.

Presentation: Poster

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Development and commercialization of predictive drug discovery platforms merging human tissue chips and translational software

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The estimation and optimization of drug properties to develop efficacious and safe therapies for humans is a critical step in preclinical discovery. Although efforts have been made to develop *in vitro* methods that deliver translational and predictive data, much testing still utilizes animal models, which create a large financial burden during lead optimization and can be inaccurate in predicting human outcomes. To address this need, we have developed an integrated platform that combines human tissue chips with translational software to predict clinical parameters of investigational drugs.

Our single- and multi-tissue chips are designed to generate multi-scale (media- and tissue-based) data by incorporating media recirculation in a COC-based milli-fluidic chip with larger media volumes (> 1ml) and tissue sizes (> 200K cells per tissue) than other microfluidic chips.

Liver, kidney, and muscle tissues on single- and multi-tissue chips were evaluated both for tissue viability and pharmacokinetic and safety pharmacology context of use (CoU) applications. Ad-

ditionally, transcriptomics analysis of the liver tissue demonstrated higher transcriptional similarity scores of *in vitro* human liver tissues to human liver tissues than animal liver similarity scores to human liver. Then, a diverse set of compounds were tested on each tissue and tissue-specific pharmacokinetic parameters (such as hepatic and renal clearance and volume of distribution) were measured. Then, on-chip results were extrapolated to human scale with companion software using quantitative systems pharmacology (QSP)-based algorithms to quantify predictability of each CoU application and compare to the predictability of animal models.

These studies demonstrated that the Javelin Biotech platform can deliver precise clinical predictions for several applications, and offer an alternative to, and hopefully a replacement of, laboratory animal studies for the purposes of understanding human pharmacology in an intact mammal as a surrogate for human.

Presentation: Invited

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Clinical relevance of a liver-heart microphysiological system to inform a patient risk scoring system for QT elongation

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There is a basic need to address Adverse Drug Effects (ADEs), especially in at risk or elderly populations. Tabula Rasa Health Care TRHC's risk stratification process, an approach to reduce and predict ADEs by the use of predictive risk scores and employment of drug claim data to predict various healthcare outcomes by scoring comprehensive drug regimen characteristics. However, data for these models is limited to literature, some *in vitro* models and patient data which are in limited use as they rely upon dose parameters not actual plasma concentrations. This risk stratification process comprises 5 aggregated medication risk mitigation factors [1]. Algorithms for these 5 factors are weighted and combined to compute a final MedWise Risk Score™ (MRS). The MRS™ is a predictive tool used by health care providers to determine which element of a drug regimen can contribute the most to a patient's likelihood of an ADE occurring. The utilization of human-on-a-chip systems coupled with PKPD *in vitro* models would provide organ expansive data and functional outcomes to input into these clinical models to improve outcome predictions. There is limited data for many drugs, especially for drug-drug interactions that do not interact directly with the drug target. Metabolic effects can be particularly difficult to predict. Hesperos has previously fabricated liver-cardiac systems with a serum-free, circulating medium for 14 days that reproduces aspects of the *in vivo* crosstalk between cardiac and liver organ with non-invasive readouts of cardiotoxicity for drugs and their metabolites. In this study, Hesperos has developed a Liver-Heart system with a real-time, high throughput measurement component for the evaluation of small molecule combinatory therapy and its effect on QT interval for informing the MRS model system.

Reference

[1] Cicali, B., Michaud, V., Knowlton, C. H. et al. (2018). Application of a novel medication-related risk stratification strategy to a self-funded employer population. Benefits Quarterly second quarter.

Presentation: Poster

738

Glial cell type composition in a 3D model of the central nervous system

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The translational gap between success in the lab versus success in clinical trials is an especially prevalent problem in neurological disease treatment, highlighting the need for novel model systems that allow drug testing in a human setting prior to patient exposure. Microphysiological systems containing human iPSC-derived cell lines provide this opportunity; however, it is important to ensure that these systems are comprised of cell types that accurately mimic an *in vivo* setting. In the central nervous system (CNS), glial cells, including astrocytes and oligodendrocytes, are integral to many aspects of neuronal function and can also be involved in neurological disease manifestation. To generate human BrainSim®, iPSC-derived neural stem cells (NSCs) are exposed to a variety of growth factors over a 12-week differentiation period. We tested a variety of media types to determine the optimal conditions for enhancing glial differentiation. By using qPCR, Western blot, immunohistochemistry (IHC), and other endpoints, we have generated compelling evidence that at 12 weeks of age, BrainSim® cultured under different media types consists not only of NSCs and neurons, but also astrocytes, oligodendrocyte precursor cells (OPCs), and oligodendrocytes with variable expression based on the media type. The appearance of markers such as GFAP, PDGFRα, and Olig2 make it possible to track the emergence of these cell types at 6 time points over the 12-week period. In the future, this will enable the testing of pharmaceutical drugs for their effects on glial development, proliferation, and differentiation, and due to

the richer cellular landscape, allow for more thorough investigation of neuroprotective and neuroregenerative targets.

Presentation: Poster

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Multi-organ on chip platforms for individualized studies of human pathophysiology

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For several decades, tissue engineering has been evolving in response to the growing needs to repair or replace our tissues and their functions lost to injury or disease. Many of the advances in this burgeoning field are driven by the increasing life expectancy and focus on healthy aging. The classical paradigm of tissue engineering involves an integrated use of human cells, biomaterial scaffolds (providing a structural and logistic template for tissue formation) and bioreactors (providing the molecular and physical signals and insights into biological events). Importantly, this biomimetic approach can be designed to provide the milieu of tissue regeneration or disease, extending the application of engineered tissues from regenerative medicine into modeling of diseases and the development of new therapies.

A reverse paradigm has emerged in recent years, with the development of microphysiological systems or "organs on chip" with functionally connected micro-sized human tissues engineered using the principles established for engineering of their clinically sized counterparts. While small and relatively simple, these tissues can be designed to recapitulate organ-level functions, such as contractility of the heart, metabolism of the liver, or barrier function of the lung or skin. For physiological relevance, the individual tissues need to maintain their individual phenotypes for weeks to months, while being allowed to communicate by secreted factors, extracellular vesicles and circulating cells.

A new class of "organs on chip" platforms is designed to maintain each tissue in its own optimized niche, link tissues by vascular flow containing circulating cells, and separate the intratissue and intravascular spaces by endothelial barriers. Engineering tissues from blood derived pluripotent stem cells allows individualized approach to biological and medical research. To illustrate the capabilities of these multi-organ platforms and some current challenges and opportunities, we discuss here a range of patient-specific studies of systemic conditions, including the injury by radiation and ischemia, infection, and cancer metastasis.

References

[1] Ronaldson-Bouchard, K. and Vunjak-Novakovic, G. (2018). *Cell Stem Cell 22*, 310-324,.
[2] Vunjak-Novakovic, G. et al. (2021). *Cell 184*, 4597-4611.
[3] Ronaldson-Bouchard, K. et al. (2022). *Nat Biomed Eng 6*, 351-371.

Presentation: Invited

740

Cosmetics Europe LRSS project: Use of skin-based multi-organ MPS models in the safety assessment of cosmetics ingredients

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The safety assessment of cosmetic ingredients registered in Europe now relies on the use of alternative methods due to the European ban on *in vivo* studies since 2013. The "Multi-Organ-Chip" (MOC) project was part of the Cosmetics Europe Long Range Science Strategy program, which ended in December 2022. This project evaluated the use of microphysiological systems, specifically the TissUse HUMIMIC Chip models, as a partial replacement for the standard *in vivo* rodent toxicokinetics studies. The main route of exposure to cosmetics ingredients is via the skin; therefore, studies investigated the combination of skin-liver Chip2 models, which allow route-specific effects and a direct interaction between these organoids to be measured in a single assay. The final stage of the project involved the incorporation of a third organoid – thyroid follicles – to investigate potential endocrine disruption of topically applied ingredients via thyroid hormone perturbation. The ultimate aim is to link biological effects of ingredients with systemic concentrations to establish a margin of safety and determine safe concentrations in the final formulations used by consumers. This presentation will address some of the common questions regarding the use of microphysiological systems in the safety assessments of cosmetic ingredients using results and key findings from the MOC project.

Presentation: Invited

741

Bioluminescent assays for monitoring cell health in microphysiological systems

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Microphysiological systems (MPS) using miniaturized 3D cell cultures and incorporating fluid flow provide improved physiologically relevant experimental models to monitor parameters not possible using monolayers of cells cultured on plastic. It is possible to measure changes in the number of viable and dead cells in MPS as internal controls using highly sensitive (< 10 cells) kinetic real-time bioluminescent assays that can be multiplexed. However, with increasing complexity of the experimental model comes a greater responsibility to characterize the cell health and physiological relevance of the model system beyond whether the cells are alive or dead. Here we describe a panel of bioluminescent assay methods for monitoring key metabolic pathways including glycolysis, the pentose phosphate pathway, fatty acid metabolism, amino acid metabolism and the TCA cycle. Appropriately functioning metabolic pathways enable cells to grow, maintain their function and response to internal and external signals. Monitoring changes in those pathways provide a valuable approach for studying cellular responses in different MPS. The luminescent metabolite assays use a common core technology that couples metabolite-specific dehydrogenase enzymes with NAD(P)H production and generation of a signal that can be recorded using a plate reading luminometer. The high sensitivity (femtomole detection limit), wide linear range (> 3 logs) and broad assay window (maximum signal above background > 100 fold) of the bioluminescent assays allow simultaneous analysis of multiple metabolites from a small amount of culture medium removed from the MPS device.

Presentation: Poster

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Evolving role of investigative toxicology in the pharmaceutical industry

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In recent years nonclinical toxicology evolved from a descriptive to a science driven investigative/mechanistic discipline. To obtain a detailed assessment of the adoption, range, and impact of investigative toxicology (I-Tox) in supporting drug discovery and development in the pharmaceutical industry, two surveys of 14 pharmaceutical companies were conducted several years apart. The survey results, together with case studies, reveal that I-Tox focus has evolved from supporting small molecule programs to a growing need for experimental support of newer drug modalities. Common deliverables of I-Tox include target safety assessment, hazard profiling of leads (*in silico*/*in vitro*) and in-depth hazard qualifying/quantifying experiments that support lead optimization and drug candidate selection prior to *in vivo* studies initiation. *In vitro* assays are commonly used for hepatotoxicity and cardiovascular toxicity profiling; however, there is a crucial need to improve the *in vitro* to *in vivo* translation. New technologies and associated models/assays such as organs on a chip, stem cell derived systems, advanced imaging methods, advanced molecular and cellular models microphysiological systems, genome editing, and quantitative modelling approaches are impacting drug design, selection, and progression. During preclinical toxicity studies, I-Tox is key to understand the mode of action of toxicities observed and their relevance to humans. Furthermore, a deep understanding of the mechanism that underlies such toxicities together with an estimate of its human relevance may allow a project to continue. Finally, we emphasize the importance of collaboration between pharma, academia, service, and technology providers to further enhance our ability to discover and develop safe medicines.

Presentation: Poster

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Dorsal root ganglion tissue model of acute and chronic nociception using hiPSC co-cultures on microelectrode arrays

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Background and justification: Chronic pain is the U.S.'s #1 public health crisis: with high incidence and no viable treatment options. Current analgesic screening methodologies based on the identification and exploitation of "druggable" targets has proven low-throughput and ineffective. Therefore, there is an urgent need for novel drug classes that may mitigate (i.e., analgesics) or even reverse chronic pain phenotypes. However, current preclinical cell- and small animal-based models and are problematic due to poor relevancy and lack of phenotypic readouts.

Methods: Here, we report on novel 2D and 3D models of acute and chronic nociception based on human induced pluripotent stem cell (hiPSC) co-cultures and microelectrode arrays (MEAs). hiPSC-derived sensory neurons and glia are co-cultured at a 2:1 ratio on multi-well MEA plates in the presence or absence of inflammatory cytokines (TNF-α and IL-6). Spontaneous and temperature-evoked activity is recorded with and without cytokines and following addition of 15 cherry-picked, "off-label" FDA-approved compounds (10 μM). Additionally, high-frequency calcium oscillations are measured from glial cells to further describe the culture's phenotypic state.

Results: 24 h incubation with inflammatory cytokines results in significant increase in spontaneous mean firing rate (MFR) as well as a significant increase in temperature responsiveness. Cytokine-treated hiPSC co-cultures serve as an "excellent" assay based on modified Z' assay quality metric (0.52) using lidocaine as a control compound. A majority of cherry-picked compounds (73%) registered as "hits" using our assay and a threshold of 3x the median absolute deviation (MAD) of cytokine-treated activity recordings. Likewise, stimulation of glial excitability resulted in "excellent" assay scores (0.63) and was used to evaluate "hits" against glial excitability.

Discussion and conclusion: In total, these data suggest that hiPSC sensory co-cultures on MEAs serve as an excellent assay for detecting compounds capable of reversing inflammatory nociception. Additionally, this same culture platform can be used to evaluate glial excitability for the purposes of differentiating effects on neurons versus glia in a heterogeneous cell population. Future work will focus on the integration of 3D MEAs and non-cherry-picked screening efforts for preclinical analgesic discovery.

Presentation: Poster

744

Engineering organoids

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Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their *in vitro* counterparts hinders their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing next-generation organoids that are assembled by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments. I will also present recent examples of how we are using these engineered organoids for disease modeling, in particular in the area of solid tumors.

Presentation: Invited

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Microphysiological systems (MPS) models of endometriosis and adenomyosis

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The endometrium is a complex mucosal barrier that lines the uterine muscle and undergoes a hormonally-driven scarless regeneration process each month. This process is also a source of illness for an estimated 200 million women worldwide who suffer debilitating pain and infertility from chronic diseases in which the endometrium grows ectopically, in the myometrium (adenomyosis) or outside the uterus, invading deep into abdominal organs and migrat-

ing throughout the body (endometriosis). Ectopic lesions undergo cyclic hormonally-induced changes that cause local tissue breakdown and bleeding. The trapped wounded tissue causes inflammation, leading to progressive invasion and fibrosis and growth of lesions from small (~0.1mm) epithelial acinar structures with associated stroma, to large (~cm) fibrotic lesions. We describe here complementary MPS models of the eutopic endometrium, which is deranged in endometriosis, and of early-stage lesions. First, we show that a completely synthetic PEG-based hydrogel supports weeks-long co-culture of encapsulated primary human endometrial epithelial organoids and stroma in static droplet culture, responding to inflammatory cues by developing features of the clinical condition termed "progesterone resistance". The PEG gel also supports formation of perfusable microvascular networks in the central region of a microfluidic device flanked on either side by fluidic channels through which medium is continuously pumped from encapsulated endothelial cells and fibroblasts. Endometrial organoids and stroma added to the initial endothelial-fibroblast gel encapsulation mixture at the time of injection form lesion-like structures in the microvascular bed. Finally, endometrial epithelial-stromal cocultures in PEG gels undergo morphogenesis into a mucosal barrier-like structure with gland- lumen features and separately-accessible apical and basal compartments when they are cultured in the tissue region of the "lesion" device, if the organoids are concentrated at one of the interfaces by gravity sedimentation at the gel-medium interface during gelation. These MPS together allow comparison of the spectrum of behaviors exhibited by control and diseased donors under conditions mimicking eutopic and ectopic endometrial microenvironments.

Presentation: Poster

746

Dissecting mechanisms of host-pathogen interaction in organ-on-chip

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The interaction of the microbiota with its host is crucial in the regulation and maintenance of physiological conditions of the human body. Deregulation of the host response and disbalance of microbial composition are directly associated with the development of various diseases, including acute and chronic infections. However, much of our knowledge about the microbiome and its impact on human health is based solely on descriptive and correlative studies. Current *in vitro* models lack the required complexity with significant limitations for the long-term coculture of a living microbiota, whilst animal models have limitations in the translation to the human situation since the composition of the microbiome and the immune system considerably differs from the human situation.

Microphysiological systems (MPS) have yet emerged as an attractive new platform for the coculture of a defined living microbiota with bioengineered tissue models recapitulating organotypic functions under precisely controlled conditions. Immunocompetent organ models can simulate critical aspects of the human immune response to microbial colonisation or infection and allow studies with a scalable biological complexity up to the emulation of cross-communication between multiple organs.

In his talk, Dr Mosig will present data on how his group is leveraging MPS of gut, liver and lung to study commensals and microbial-derived metabolites and how they can protect from infections. He will present examples from viral, bacterial and fungal infection studies and approaches to proving causative links to the onset of chronic recuring infections and dysbiosis-associated diseases by considering the impact of an organotypic microenvironment in MPS.

Presentation: Invited

747

Advancing new alternative methods at FDA

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FDA has a long-standing commitment to promote the development and use of new technologies to better predict human and animal responses to substances relevant to its regulatory mission. Drs Fitzpatrick and Mendrick chair FDA's Alternative Methods Working Group comprised of regulators and researchers from each regulatory Center, Office of Regulatory Affairs, National Center for Toxicological Research, and the Office of Chief Scientist. All centers have active research programs in using alternative methods that includes approaches such as microphysiological systems and *in silico* approaches. The discussion today will focus on the research being done at FDA in advancing new methods for safety and efficacy.

Presentation: Invited

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Ethical considerations in obtaining human cells for multi-organ microphysiological systems research

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The primary ethical justification for microphysiological systems (MPS) research relates to its promise to enhance scientific understanding across a wide spectrum of topics using a variety of novel techniques. This justification is bolstered by the promise of decreasing the use of non-human animals in research. Nevertheless, MPS research can also involve surprisingly complex ethical issues. At a fundamental level, these issues include assessing the potential moral status of particular MPS, such as sophisticated embryo models (e.g., synthetic embryos, blastoids) and brain organoids/assembloids. The complexity and extent of maturation of these MPS can also be of moral relevance. Furthermore, beyond standard ethics considerations for basic and translational research, proposed MPS research uses can raise moral concern (e.g., some work with chimeras) and should be addressed. Regardless, the source and provenance of the human cells used in MPS research always warrants careful deliberation that assimilates these fundamental considerations. Of primary importance here is the appropriateness and consistency of collection process, including consent, with the proposed MPS research. Policies and guidelines issued by scientific societies (e.g., the International Society for Stem Cell Research, HYBRIDA) can provide useful frameworks to facilitate these analyses. Ideally, they are informed by incorporating relevant and systematic perspectives of patients and the public. In addition, independent oversight by properly constructed research ethics committees, stem cell research oversight committees and animal care and use committees can help assess these issues while also facilitating compliance with applicable regulations and policies. For MPS research to meet its promise, the associated ethical issues must be addressed.

Presentation: Invited

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Organotypic chip models and applications in disease studies

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Organ-on-Chip and organoid models are next generation platforms for studying human biology and disease in an organotypic context. However, in order to develop and test robust protocols, it is important to define what "organotypic" means. In our team, we derive structural and functional metrics from native human organ tissues and develop imaging tools, computational models, microfabrication and culturing strategies to assess and match these metrics *in vitro*. The standardized and quantifiable representation of the healthy phenotype allows for better detection and matching of disease relevant phenotypes, as we have shown for liver and lung models. We especially leverage mechanical cues to fine-tune self-organization and polarization of tissues. For example, we achieved the formation of organotypic bile canaliculi networks in a liver chip model using optimized matrix scaffolding. This chip design was then used to recapitulate alcohol toxicity-induced cholestasis seen in the clinic (Nawroth & Petropolis et al., Cell Reports 2021). Here, I will give an overview of our technology development goals and discuss our recent insights into engineering organotypic tissue models and their applications in disease studies.

Presentation: Invited

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The state of the MPS revolution

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Human stem cells have finally made all human cell types available for biomedical models, with optimization of their maturation and ageing increasingly reflecting all life stages. Bioengineering has enabled moving from keeping them alive to actual functional units. The addition of perfusion has introduced homeostatic conditions and the possibility to combine organ units. Together this enables to realize physiology in small models, aka MPS. Increas-

ingly, organ functionalities become relevant benchmarks of these systems. The next step is the modeling of pathophysiology, the Micro-PathoPhysiological Systems (MPPS). There are three principal ways to disease – genetics, pathogens and exposure. MPPS allow to study them all and their combinations. They more and more outperform animal models, avoiding species-differences, restricted genetic variability, statistical underpowering etc.

To bring M(P)PS to their full potential, we need a thriving multi-disciplinary community to exchange ideas. We need to prove the quality of these approaches by best practices, reporting standards and validation. We have to ensure the availability and access to this technology by teaching offers at all stages of career, standardization and a biotech ecosystem of contract research, devices, models, consumables, and measurement technologies. Together, they promise a scientific revolution, where human inter-individual differences can be studied in health and disease and form the basis of the development of drug and other interventions, as a truly disruptive technology.

Presentation: Invited

751

Advancing pre-clinical safety assessment with MPS: The road to model qualification and adoption

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Enhancing the early detection of safety liabilities of new therapies would advance drug discovery. Advanced human cell models, including microphysiological systems (MPS) aim to recapitulate tissue architecture, cell-cell interactions, and cell microenvironment, making them more representative of complex *in vivo* biology than standard two-dimensional culture. Thus human MPS provide an opportunity to improve preclinical-to-clinical translation.

In Clinical Pharmacology and Safety Sciences at AstraZeneca, we are developing advanced human cell models for a number of different organs and safety applications, depending on the context of use. This presentation will highlight several examples, demonstrating how the data generated from these systems are being used to improve pre-clinical safety assessment and how these models are enhancing the human-relevance of our pre-clinical tests.

Examples will include human 3D static and fluidic (MPS) bone marrow (BM) models and a human kidney proximal tubule-on-a-chip (PT MPS). Our static/MPS BM models maintain stem cells with concurrent differentiation into erythroid, myeloid and megakaryocyte cells and are used for candidate drug selection or to guide oncology drug combination-doses and drug-sequencing/schedules. Antisense oligonucleotides (ASOs) are increasingly being developed as a therapeutic modality, however, preclinical safety evaluation of ASOs lacks predictivity due to the simplicity of *in vitro* models and a lack of cross-species reactivity *in vivo*. To address these challenges, we have developed a PT MPS, which has a polarised epithelium and recapitulates transporter-mediated drug toxicity. Clinical translation of these models is critical for accurate safety assessment. Our strategy is to integrate quantitative systems toxicology modelling with our advanced cell models, and examples of how we are successfully implementing this will be presented.

While advanced cell models have the potential to enhance the human-relevance of pre-clinical safety assessment, there remain challenges to their adoption and development in the pharmaceutical industry, including a lack of standardisation, high cost and time-requirement, and low sample volumes/cell numbers for downstream analyses. This presentation will also address these challenges and present opportunities to further enhance advanced cell models and thereby drive their adoption.

Presentation: Invited

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Unlocking the potential of organoid and tissue models for drug discovery with platform technology

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Despite annual spending of over 100 billion dollars on drug development, the success rate from phase I to market approval remains under 10%. Late-stage clinical trial failures make the drug development process inefficient and extremely costly. Therefore, even a small improvement in the predictive capacity for selecting successful drug candidates at the pre-clinical trial stage could translate to multi-billion dollar savings. The preclinical drug discovery process has relied on animal models and cell-based assays for decades, and there is a pressing need to modernize this approach with new tools and processes. Our lab has developed bioengineering platforms that integrate stem cell-derived organoids, organ-on-a-chip technology, and machine learning to move towards an automated high-throughput and high-content drug discovery process. In this seminar, we will discuss our IFlowPlate platform, which enables the vascularization of organoids to study tissue inflammation and model the barrier function of various tissues with robotic automation. We will also present our AngioPlate platform, which allows the construction of complex 3D perfusable tissues in 384-well plate formats. Our aim is to combine these individual efforts to develop a more

precise and integrated high-throughput and high-content drug discovery process for drug screening and target validation. With our approach, we hope to modernize the drug discovery process, leading to more effective drug development and ultimately benefiting patients worldwide.

Presentation: Invited

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Advancing acceptance of MPS for regulatory testing of medicinal products in the EU

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The European Medicines Agency (EMA) has a long-standing commitment towards the application of the principles of Replacement, Reduction and Refinement (3Rs). This is driven by the requirements of Directive 2010/63/EU, as well as by the crucial need for better tools to predict quality, safety and efficacy of new medicinal products.

EMA's regulatory science strategy 2025, clearly recommends the leverage and qualification of 3R testing approaches or Novel Approach Methodologies (NAMs). It recognises the need for discussion on and definition of regulatory acceptance criteria (e.g. context of use, endpoints and reference compounds) in order to promote regulatory acceptance of NAMs. For this engagement with stakeholders is seen as instrumental.

Recently, a new 3Rs Working Party (3RsWP) has been set up as the official 3Rs hub for at the EMA. The 3RsWP is initiating a broad set of activities dedicated to the qualification of NAMs or 3R testing approaches. These include the organisation of workshops on MPS and organ-on-chip with a specific focus towards method qualification, the definition of regulatory acceptance criteria for organ-on-chip technologies for specific contexts of use and the initiation of an international regulatory conversation in order to harmonise views and acceptance criteria.

Specific challenges and opportunities related to the regulatory acceptance of MPS systems for the testing of medicinal products will be addressed. These include the conclusions from the first EMA workshop on non-animal approaches in support of medicinal product development. Focus will be drawn to the importance of context of use in the setting of qualification criteria, and the identification of reference compounds and performance standards.

Presentation: Invited

754

Can engineered organotypic models predict patient-specific response?

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In vitro cell-based assays for the prediction of patient-specific cancer response have not been widely adopted. However, it is timely to reevaluate their use, as numerous innovations, including micro-scale organ-on-a-chip models, may improve their predictive power and utility and make them a more attractive alternative to animal models. We are exploring how different levels of organotypic complexity may be leveraged to recapitulate patient response in different disease states. Progress and challenges towards generating patient-specific microphysiological systems will be discussed as well as results demonstrating the models' ability to respond to various treatment regimes. The tradeoffs between the model constraints for clinical use vs. mechanistic studies as well as the challenges of moving these models into the clinic will also be discussed.

Presentation: Invited

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Novel models and technologies for developmental and adult neurotoxicity prediction

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The fields of repeat-dose organ toxicity (e.g. targeting the nervous system) and of developmental toxicity affecting brain structure and function (DNT) are considered particularly difficult. Due to their complexity they have often been considered unsuitable for non-animal new approach methods (NAM). We set out to develop human cell-based predictive assays in these fields. The test systems employed range from conventional 2D cultures, over layered or self-organized co-cultures to 3D organoids. The setup and complexity have been adapted to the requirements of toxicological data generation. While throughput was given priority for some endpoints, complex functional endpoints, or high content (multi-dimensional) information was preferred for others. An *in vitro* test battery (IVB) for DNT testing will be exemplified. For acute neurotoxicity, the examples given will range from simple neurite degeneration assays, over receptor function and respiration capacity tests towards multi-omics endpoints. While all major cell types of

the brain are available for testing already now (neurons, macroglia, microglia), their sub-specification (e.g. sensory neurons vs cortical neurons), their ratios (e.g. astrocytes vs neurons) and their spatial distribution (2D vs 3D aggregates vs structured neural tissue) will be highlighted as future challenges.

References

[1] Suciu, I. et al. (2023). Dynamic metabolic and transcriptional responses of proteasome-inhibited neurons. *Antioxidants 12*, 164.

[2] Blum, J. et al. (2023). Establishment of a human cell-based in vitro battery to assess developmental neurotoxicity hazard of chemicals. *Chemosphere 311*, 137035.

[3] Holzer, A. K. et al. (2022). Specific attenuation of purinergic signaling during bortezomib-induced peripheral neuropathy. *Int J Mol Sci 23*, 3734.

[4] Loser, D. et al. (2021). Acute effects of the imidacloprid metabolite desnitro-imidacloprid on human nACh receptors relevant for neuronal signaling. *Arch Toxicol 95*, 3695-3716.

[5] Meisig, J. et al. (2020). Kinetic modeling of stem cell transcriptome dynamics to identify regulatory modules of normal and disturbed neuroectodermal differentiation. *Nucleic Acids Res 48*, 12577-12592.

[6] Brüll, M. et al. (2020). Incorporation of stem cell-derived astrocytes into neuronal organoids to allow neuro-glial interactions in toxicological studies. *ALTEX 37*, 409-428. doi:10.14573/altex.1911111

Presentation: Invited

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A bioengineering approach to T cell diversity

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It is believed that immunity mainly occurs in tissues, not in blood. Nevertheless, blood samples and derived systems remain the most common human-based models for fundamental studies and drug profiling due to practical considerations and lack of superior alternatives. HOPE aims at bioengineering the next generation of interconnected, functional lymphoid and non-lymphoid tissues capable of mounting innate and adaptive immune responses to antigens and new molecular entities, and thereby doubling the predictive value of preclinical studies for immunotherapies. To achieve this, HOPE leverages universal stem cell models and donor-derived primary systems to reach unprecedented levels of immune diversity, specificity and functionality while ensuring scalability and robustness across platforms. As HOPE teams progressed towards these goals in the past 2 years, knowledge and technical gaps were identified and, sometimes, resolved. In particular, HOPE dedicated efforts for depicting the TCR repertoire of matching donor-derived blood and tonsil samples at an unprecedented level of coverage. Our 10M T cell study will provide the deepest characterization of the TCR repertoire found in healthy human blood and tonsils reported thus far, and help define the guiding principles for bioengineering microphysiological systems predictive of human immune responses.

Presentation: Invited

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Application of MPS to ADME studies: *In vitro* model for intestinal drug absorption

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Prediction of pharmacokinetics of drugs in human body is important issue for development of new chemical entities. To address the species difference, human derived materials have been used for drug metabolism and transport studies. Nowadays, human cryopreserved hepatocytes, or freshly prepared human hepatocytes as well as human liver microsomes are most frequently used for this purpose. The intestine serves as site for drugs orally administered, and its fraction of absorbed and availability are key factor for successful development of oral drugs including ester-type prodrugs. We first introduced Ussing chamber system with freshly isolated human intestine, however, it is not appropriate model for routine assay. Recently, we introduced the technique to prepare spheroids from intestine crypt of various segments of human and animals, and after expansion, they are differentiated to the absorption epithelial cells in the cell-culture insert. The epithelial cells showed the CYP3A4 activities that could reasonably account for *in vivo* availability of CYP3A4 substrates in humans. Furthermore, they also maintain the transport activities of P-gp and BCRP which are well characterized in the intestine. We can successfully prepare the spheroids from various regions of the intestine to investigate regional dependence of the drug absorption. The spheroids and organoids can also serve as *in vitro* model of drug effect. Among the MPS project in Japan, we are trying to establish the intestine models using microfluidics.

Reference

Michiba, K. et al. (2022). *Drug Metab Dispos 50*, 204-213.

Presentation: Invited

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Initiatives for new approach methods at Japanese Center for the Validation of Alternative Methods (JaCVAM)

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Japanese Center for the Validation of Alternative Methods (JaCVAM) is one of the facilities of the Center for Biological Safety and Research (CBSR), NIHS, which cooperates with various related facilities within and outside Japan to conduct activities to promote the use of alternative methods for animal experiments. With the support of the International Cooperation on Alternative Test Methods (ICATM), JaCVAM has participated in more than 20 validation studies and contributed to the development of more than 15 test method guidelines (TGs) developed by the Japanese in the field of human health in the OECD. There are 75 TGs in this field in the OECD, so this means that the JaCVAM has contributed to the officialization of 1/5 of all TGs in the 16 years since its establishment. Although most of these TGs are limited to local toxicity studies such as eye irritation and skin sensitization, this international experience is an invaluable asset and must be utilized in the development of future TGs.

In this presentation, I would like to introduce our current activities as Chair of the Steering Committee of JaCVAM, including the development of a new approach methods (NAMs). For example, JaCVAM is working on the officialization of the Hand1-Luc Embryonic Stem Cell Test (for developmental toxicity) and the IL-2 Luc assay (for immunotoxicity) as alternative methods for general toxicity testing. We are also supporting the development of Physiologically Based Kinetic models, Micro-physiological System, Artificial Intelligence based Substance Hazard Integrated Prediction System (AI-SHIPS), so on, and their combination for NAMs. We would like to accelerate the development of the NAMs by deepening cooperation with ICATM and other experts in this field in Asia and Oceania.

Presentation: Invited

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Reconstructing metabolic cross talk on chip

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Modelling organ(oid) cross talk *on chip* calls for a robust and scalable platform that allows connecting functional tissue units without the need of external support systems. We developed a novel, recirculating organ-on-chip (rOoC) platform that creates a controlled unidirectional gravity-driven flow by combining a 3D-tilting system and an optimized microfluidic layout. The rOoC platform allows to grow and connect two or more tissue or organ representations *on chip* with the possibility of applying endothelial barriers, microvasculature and circulating cells such as immune cells, independent of external tubing and support systems. In parallel, we developed human pluripotent stem cell (PSC) derived islet organoids, and liver organoids both showing close to mature functional properties including a zone-specific identity of the liver organoids. The rOoC platform supports long-term culture (for at least 2 weeks) of islet and liver organoids and allows functional interrogation of the integrated organoids under physiological and diseased conditions.

Presentation: Invited

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Mapping the development and regeneration of reproductive tissues

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The study of reproductive tissues requires a systems biology approach. Their development starts in utero and does not finish until after puberty, leading to changes in organization and cell composition across the lifespan via paracrine (e.g. folliculogenesis) and endocrine (e.g. menstrual cycle) signals. Our team has generated a comprehensive map of human developing and adult reproductive tissues using a combination of single-cell and spatial transcriptomics, chromatin accessibility assays and fluorescent microscopy. We

utilise this map to study the aetiology of reproductive disorders, identify novel therapeutic targets, and guide the development of *in vitro* models

Presentation: Invited

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"The Sound of Safety" – combining MPS with Bio-AI and *in-silico* to capture the signature of the ordinary (non-toxic) behavior of MPS and the deviations under increasing concentrations of drugs

Yossi Haran[1], Amir Bein[2] and Shahar Harel[1]

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Failure to faithfully predict safety of drugs in humans is a major problem negatively affecting drug development, leading to tremendous waste of time and money. In the past decade, there have been significant breakthroughs in the fields of microphysiological systems (MPS) and organs-on-chip with a focus on better prediction of response to drugs in humans. This, in turn, led to numerous commercialized MPS solutions, most of them focusing on efficacy of new drugs as well as addressing the safety prediction challenge. To further improve the clinical prediction capabilities, we developed a unique Hybrid, Bio-AI approach introducing a new tool that harnesses the human physiologically relevant responses of a liver model in combination with a strong artificial intelligence (AI) engine trained on millions of interactions. between the liver model and known drugs and merged with world knowledge and datasets of physicochemical properties.

Putting our Hybrid model to a test, we found it can outperform published data of MPS systems alone. Furthermore, it was successful at correctly predicting the DILI level of 4 blinded drugs in a recent unpublished study.

In this talk, we will also present an innovative concept and initial promising results for predicting the safe dosing window based on training a machine learning (ML) model to identify responses of MPS organ models to compounds known as safe and categorize them as "typical behavior" or safe. Once the model is trained on a large enough set of safe drugs in safe doses, the model can distinguish between the normal response and abnormal ones. When a new drug is tested on this platform at increasing concentrations, the system can identify a non-characteristic response based on the training set.

Presentation: Invited

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Comprehensive tumor modelling and its application in discovery and development of next generation oncology drugs

Karla Queiroz[1], Orsola Mocellin[1], Abbie Robinson[1], Flavio Bonanini[1], Sander de Ruiter[1], Vincent van Duinen[1], Arthur Stok[1], Gilles van Tienderen[2], Monique Verstegen[2], Aleksandra Olczyk[1], Stephane Treillard[1], Thomas Olivier[1], Chee P. Ng[1], Jeroen Heijmans[1], Henriëtte L. Lanz[1], Jos Joore[1] and Paul Vulto[1]

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Its incidence is increasing and is closely related to advanced liver disease. Interactions in the HCC microenvironment between tumor cells and the associated stroma actively regulate tumor initiation, progression, metastasis, and therapy response.

Here we present a study on comprehensively modelling of the tumor environment and deploying these models for drug response testing and screening. The models comprise a full vascular plexus, stroma, immune cells and hepatocellular carcinoma material from 8 different donors. Cultures were exposed to a panel of drugs or drug combinations and were analyzed in terms of their viability (Alamar blue assay), and chemokine/cytokine levels in the supernatant (Luminex). In addition, the organization of the vasculature in the tumor compartment was studied through immunostainings, confocal imaging, and subsequent morphological analyses. From these data we could derive patient-specific responses following a range of parameters, leading to selection of most promising (combination) treatments.

We envision that this patient derived model will evolve to become a platform for understanding the interplay between angiogenesis, stroma and immune infiltrate in HCC and will be particularly useful for developing novel immune therapeutic treatments.

Presentation: Oral

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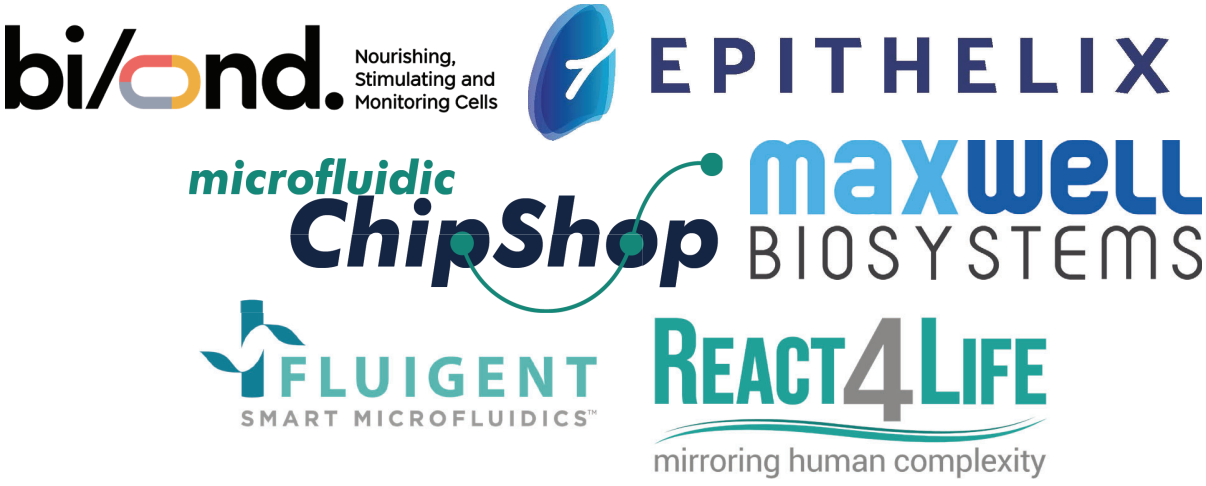

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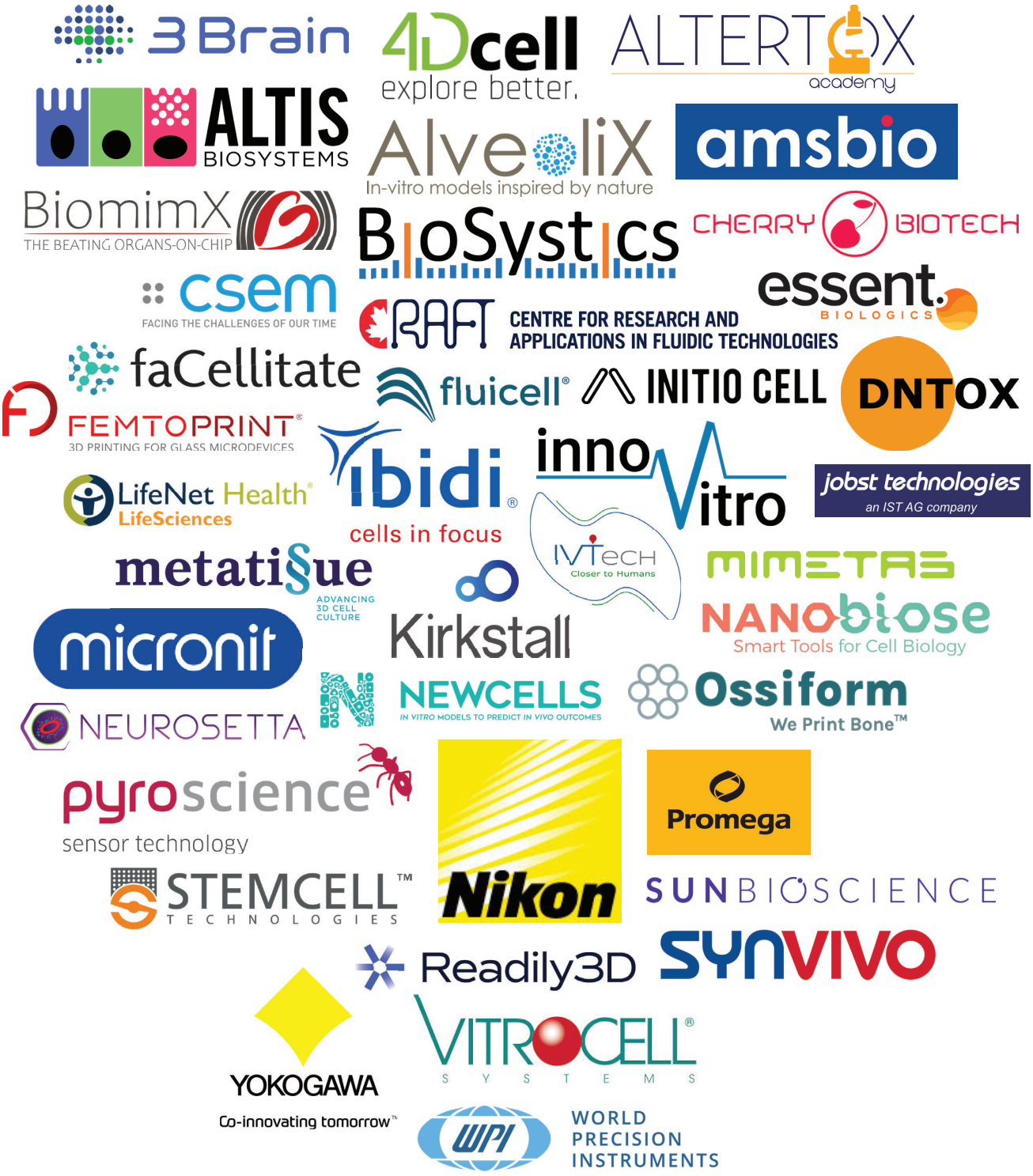
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Vol. 11, No. 1 (2023). doi:10.58847/ap.2301

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