Abstracts of the 1st Microphysiological Systems World Summit, New Orleans, 2022

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Dear colleagues, collaborators, and friends,

Something big is happening – a vanguard of cell biologists, biomedical researchers, and bioengineers are meeting to formalize their international collaborations and exchange their ideas and experience in creating a new generation of models of human and animal organs and organisms. The FDA coined the term microphysiological systems (MPS), but access to human cells and tissues has been limited, and continuous access to high-quality cells is a major challenge. The marriage of stem cell technology and bioengineering has brought about the realization of complex tissue architecture and functionality in human *in vitro* systems. These revolutionary changes in cell and tissue culture are making more and more human-relevant models available for the life sciences. Technologies are maturing, their broad availability is improving, and they are broadly impacting science.

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Suzanne Fitzpatrick
U.S. Food and Drug Administration

Donald E. Ingber
Wyss Institute, Harvard University

Lena Smirnova
Johns Hopkins University

Camila Januario
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Three years ago, Dan Tagle, NIH NCATS, and I discussed over a beer at our microphysiological systems (MPS) workshop in Berlin (Roth and MPS Berlin, 2019; Marx et al., 2020) that the field needs a series of international meetings and a society. With his vision and support, this has now become a reality. About sixty organizations have joined us to form a steering group for this series of conferences and the creation of an International Society. This steering group, complemented by a scientific advisory committee of individual experts, has enabled a bottom-up process to make this happen. Early on, my co-hosts Suzie Fitzpatrick, FDA, and Don Ingber, Harvard Wyss Institute, joined these efforts and were keynote speakers of our two virtual pre-conferences attended by about 700 people. They have given this initiative face and direction. A number of committees have dedicated enormous time and effort to fundraising, program development, local organization, and international society formation. The preparation of the next MPS World Summit in Berlin from 26 to 30 June 2023 is already underway.

The New Orleans kick-off promises to be a remarkable gathering of pioneers of these new technologies: 137 speakers (56 from abstracts/81 invited), 171 posters, and approaching 600 registrations at the time of writing from all parts of the world show the enormous interest of the scientific community. Bringing the series of world summits to Europe in 2023 and then to the US West Coast in 2024 with the goal to further expand into the Asia-Pacific area will further enhance this.

This New Orleans event wants to be not just another scientific meeting of presentations and posters. We want to enable discussion and interaction. Starting with a Creole Queen Dinner Jazz Cruise as an in-

formal gathering to lead into the meeting, a Welcome Reception on our first day, a guided city tour, our Macro Party, and the Jones Walker Sunset Reception, there is ample opportunity to meet and talk beyond the catered combined poster and industry exhibition.

Endless hours were spent by our team led by Lena Smirnova with respect to science as well as Camila Januario and her administrative team. This is not a meeting organized as one of many by a seasoned organization – it is done with heart blood and enthusiasm by a large group of people from all over the world, who felt that it is time to come together and mark that *in vitro* research is changing.

Let's make this a remarkable milestone in changing the landscape of biomedical research.

Thomas Hartung

References

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Dear MPS World Summit participants,

The journal ALTEX Proceedings is honored to publish the Abstract Book of the 1st Microphysiological Systems World Summit in New Orleans, LA, USA.

As the World Summit was initiated and planned during the global pandemic of COVID-19, it was designed to be held in a hybrid format from the start. With travel restrictions having been lifted in most countries, many of you have traveled to New Orleans, eager to meet and network in this specialized scientific community. However, the hybrid format will allow persons who are not yet able to travel or do not have the funds to do so to take part in the meeting virtually and learn about current innovations and opportunities in this field.

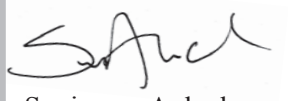
This Abstract Book contains more than 300 abstracts that have been accepted for oral or poster presentations sorted by submission ID number. They represent the work of about 1,300 contributing authors from 25 countries on five continents.

We are grateful to the Doerenkamp-Zbinden Foundation, Kreuzlingen, Switzerland for generously funding the production of this Abstract Book. We thank Lena Smirnova and Anwyn Statnick from the Center for Alternatives to Animal Testing, Johns Hopkins University, Baltimore, MD, USA for their excellent cooperation in producing the Abstract Book.

We wish all participants of the 1st MPS World Summit a successful and inspiring meeting that will initiate new collaborations in this auspicious and exciting field of research, which holds so much promise to reduce and replace the use of animals for scientific purposes and to improve the health and safety of humans.

We can already look forward to meeting again in Berlin, Germany in June 2023 for the 2nd MPS World Summit.

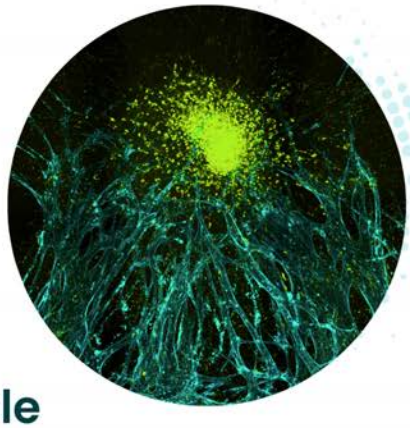
With best wishes,

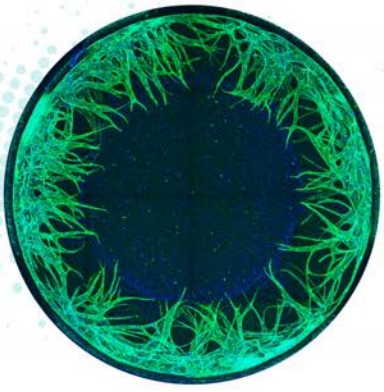


Sonja von Aulock
Editor in chief, ALTEX & ALTEX Proceedings

Qureator's Curiochips

Qureator's Microphysiological Systems (MPS), known as Curiochips, are designed for recapitulating the complexity of tissue microenvironments, thus providing ideal microscale 3D cell culture conditions for a variety of applications in biomedical research.





A.R.C.H. Principle

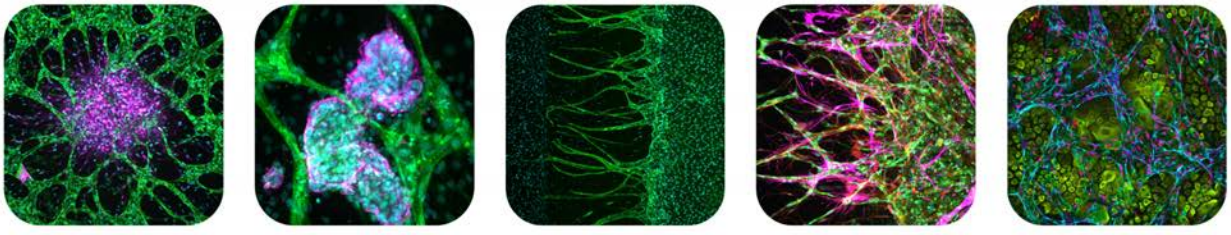
Curiochips are designed on the "ARCH" principle - Adoptability, Reproducibility, Customizability, and High-Throughput.

Qureator's Curiochips Microfluidic Technology Allows For:

- Investigation of underlying biology of disease
- Target validation in 3D co-culture conditions
- Establishment of disease models composed of patient-derived samples
- Large-scale drug screening for a new therapy or combo agents with existing drugs
- Prediction of clinical responses and patient stratification
- Identification of biomarkers for monitoring drug's efficacy in future clinical studies

Applications

Oncology (Tumor Microenvironment) | Vascular Biology | Blood-Retinal-Barrier | Dermal Angiogenesis



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Abstracts

3

Use of a dynamic skin and liver co-culture model to investigate the effect of application route on the metabolism of the hair dye, 4-amino-2-hydroxytoluene

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The Cosmetics Europe's Long Range Science Strategy (LRSS) aims to establish the link between dermal and systemic exposure route. In this project, we have used TissUse's microfluidic platform, the HUMIMIC Chip2 model, incorporating different skin models (provided by MatTek and Henkel) and liver organoids (consisting of HepaRG™ and stellate cells), to investigate the influence of exposure scenarios on the bioavailability and metabolic fate of chemicals. The aromatic amine hair dye, 4-amino-2-hydroxytoluene (AHT), was selected as a case study chemical to determine whether the Chip2 model could be used to mimic the first-pass effect in the skin that was observed in *in vivo* studies in rats. Both organoids were well maintained over 6 days, as indicated by TEER, metabolic analysis, and viability markers. The kinetics of AHT and several of its metabolites, including N-acetyl-AHT and AHT-sulfate, differed between topical and systemic application. Importantly, topical application resulted in a higher peak concentration of N-acetyl-AHT and increase of its area under the curve (AUC), demonstrating that a first-pass effect of N-acetylation in the skin had occurred. There was a concomitant decrease in the peak concentration and AUC of AHT-sulfate after topical compared to systemic application. These results were in accordance with *in vivo* observations, where the ratios of these two metabolites were altered by the application route. In conclusion, these data demonstrate that the Chip2 maintains the functions of skin and liver organoids for several days. Importantly, the Chip2 model recapitulated the route-specific alteration in the metabolite profile of AHT observed *in vivo*. This type of information is important for the risk assessment of topically applied compounds which may also undergo first-pass metabolism in the skin and whose systemic effects are altered accordingly.

Presentation: Poster

4

Machine learned vascularized networks improve predictive power of organ-chips

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Microphysiological systems have gained increasing attention for their applications in drug discovery and testing, toxicity screening, and more recently disease modeling. However, microvascular networks are rarely incorporated into these devices despite being a major component of almost all tissues of the body. Of the reports integrating these networks, many use different quantification tools and fail to relate these quantifications to the biological function of the networks. Here, we assessed 500 samples of microvascular networks-on-chips composed of different endothelial cell densities and sources, supporting cells, growth factors, and extracellular matrices. Since oxygen supplied by the vasculature is crucial for cell function and survival, we quantitated oxygen carrying capacity of the networks using a computational model, resulting in a representative value of the biological function of each sample that poorly cross-correlated to nearly all 10 available metrics used in prior literature. To address this problem, using machine learning, we have developed a neural-network model that provides a quantitative measure of a vascularized network that closely correlates to its physiological function within an organ-on-chip platform. Finally, we demonstrate the application of this machine-learned vascularized organ-on-chip by constructing a pancreatic microenvironment featuring insulin-secreting islet cells. This model successfully predicts that vascularization rescues insulin secretion when pancreas-chips are placed in hypoxia, demonstrating that the integration of microvascular networks plays a crucial role in the oxygenation and maintenance of a tissue-specific microphysiological system.

Presentation: Oral

5

3D prostate cancer model with ethnically/racially diverse PDXs

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Black men with PCa experience double the incidence and mortality rates as compared to white men. Population-based *in vitro* PCa models that support crosstalk between different cell types will improve our understanding of the underlying mechanisms contributing to this cancer health disparity (CHD) and improve the prediction of drug response in specific populations. Here, we used the high-throughput, perfusion-based microfluidic platform called the MIMETAS OrganoPlate® to culture up to 96 individual, multicellular PCa-on-a-chip cultures in parallel. PCa PDX cells of various R/E were embedded together with E2Crimson-labeled stromal fibroblasts in a migration-permissive hyaluronic acid hydrogel in the gel compartment, alongside an endothelium-lined perfusion channel containing a targeted immune cell population. Using high content imaging, we confirmed the stability and viability of the cultures over 7 days. The cultures maintained a 3D structure with PCa cells and fibroblasts evenly dispersed throughout the height of the gel compartment and a blood vessel-like structure within the perfusion channel. Closer evaluation of the gel compartment revealed a close association between PCa cells and stromal fibroblasts in a core-shell structure with PCa clusters surrounded by fibroblasts. Further, all cells maintained expected expression of phenotypic markers (PCa: epCAM, PSMA; fibroblast: vimentin, endothelium: CD31). For the immune component, medium and cell tracker dye conditions were optimized to support immune cell viability. Over 72 hours, immune cells were monitored, revealing migration of PBMCs through the gel compartment. Finally, the value of the complex, population-based multicellular *in vitro* model of PCa will be evaluated in a drug screen comparing the system with simple, PCa PDX monocultures. This R/E diverse 3D prostate tumor model will enable the full incorporation of all cell types and ECM into a single model which will better recapitulate population specific PCa and drug response.

Presentation: Poster

6

Modelling T cell-vasculature co-culture in a high-throughput microfluidic platform

Desiree Goubert, Michelle Brouwer, Sacha Spelier, Luuk de Haan, Johnny Suijker, Lenie van den Broek, Henriette Lanz, Jos Joore, Paul Vulto and Karla Queiroz

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Clinical success of immune checkpoint inhibitors and chimeric antigen receptor T cells has highly increased the attention of the field of immunotherapy. Identifying responders to these therapies is challenging and underscores the need for translational models that increase understanding of these responses. We established a co-culture system with immune cells and vasculature. Both essential components of the tumor microenvironment and often lacking in *in vitro* models.

We optimized endothelial and $CD8^+$ T cell co-cultures and assessed T cell migration from the endothelial tubes via endothelial sprouts towards various chemoattractants. To generate stratified 3D co-cultures, the OrganoPlate Graft was used for generating endothelial tubules, whilst angiogenic factors (S1P, VEGF, bFGF and PMA) were added to its graft chamber resulting in a gradient and sprouting of the endothelial tubes towards this chamber. Angiogenic endothelial tubules formed vascular beds within 3-5 days. Once the beds were formed, activated, and fluorescently labeled $CD8^+$ T cells were loaded in the endothelial tubules and followed for 48 hours. $CD8^+$ T cell migration was observed via the sprouts as well as by crossing the endothelial barrier and increased in presence of gradients of CCL2, CCXl12 and CCL9. Highest $CD8^+$ T cell numbers were observed in presence of a gradient generated with a mix of these chemokines. We present a high throughput co-culture system containing angiogenic endothelial tubules and $CD8^+$ T cells. The co-cultures are highly suitable for studying T cell migration, event which precedes the detection and recognition of antigens at the surface of antigen-presenting cells and for interactions with other cells involved in the immune response. It serves to understand the interplay between T cell migration and angiogenesis in the tumor microenvironment. We envision that this model will evolve into an immunocompetent patient-derived tumor model that can be used to study immune responses to tumors.

Presentation: Poster

7

A robotized 1546 compound screen in a perfused 3D microfluidic angiogenesis assay

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The Organ-on-a-Chip field provides better insight into healthy biology, but also disease, and drug development, through more physiologically relevant models. In drug development, Organs-on-Chips aims to improve the evaluation of compound efficacy. In addition, secondary effects and toxicity could be assessed in a more relevant context. Thereby, Organs-on-Chips contribute to multiple phases of the drug discovery and development pipeline.

Here, we report the screening of 1546 compounds on a 3D vascular endothelial sprouting model in the OrganoPlate® 3-lane 64. A plate comprises 64 chips, consisting of three channels each. In this model, the middle channel was filled with collagen-I, supporting a tube of HUVECs in the flanking channel which forms under gravity-driven perfusion. In addition, the ECM serves as scaffold for the angiogenic sprouting, induced through a gradient of angiogenic factors. Using a 1546 compound protein kinase inhibitor library, we screened for an effective inhibitor or enhancer of angiogenic sprouting. Through fluorescent staining and high content imaging, the effect of each compound on multiple sprouting parameters could be captured. In addition, potential off-target effects and toxicity were checked by assessing the morphology of the parent tube simultaneously. In conclusion, our compound screening using the OrganoPlate® 3-lane 64 yielded multiple non-toxic angiogenic modulators. To our knowledge, this effort is also the largest reported Organ-on-a-Chip screen, demonstrating the potential of the OrganoPlate® platform in drug discovery and development.

Presentation: Poster

8

Human lung-on-a-chip enabled modeling of respiratory viral infection and therapeutic discovery

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The increasing incidence of potential pandemic viruses – such as influenza virus and SARS-CoV-2 – necessitates new preclinical tools that can accelerate the development of effective therapeutics. We have developed human Airway Chips and Alveolus Chips that enable faithful reproduction of the pathophysiology during viral infection of the human upper airway and the distal lung, respectively. These models allow us to study more complex disease pathology, uncover novel drug targets, and develop treatments that will better translate into humans for this and future pandemics.

Presentation: Poster

9

Comprehensive evaluation of the effect of culture media perfusion on the reabsorption function of a proximal tubule on a chip

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The renal proximal tubule is responsible for reabsorption of water, amino acids, and other molecules from the glomerular filtrate which are not meant for excretion. For example, albumin and glucose are entirely reclaimed by a healthy organ. Numerous factors improve the reabsorption capacity of the proximal tubule epithelium such as paracrine communication with endothelial cells and shear stress (Lin et al., 2019). This study focuses on quantifying the enhancements in reabsorption rates of albumin and glucose using a microphysiological system. Our chip is comprised of an epithelial/endothelial bilayer with microfluidic channels through which cell-specific media is perfused independently.

It is established that the exposure of the apical side of the epithelium to flow-induced shear stress promotes cytoskeletal arrangements, enhances formation of microvilli, and hence improves molecular transport (Duan et al., 2008). Here we show that such stimulus not only increases the density and length of apical villi but also improves the expression levels of megalin, an albumin transporter, and SGLT2, a glucose co-transporter. The expression intensity and localization of transporter proteins were assessed by analyzing stacks of high pitch confocal fluorescent images taken from immunomarked samples. It was determined that flow activates megalin, causing the protein to move into the cytosol, consequently improving uptake of albumin (Dickson et al., 2014). Z-intensity profiling also made clear that the membrane-bond SGLT2 becomes distant from the nucleus due to shear stress. This in turn is evidence for a squamous to cuboidal morphology translation of the epithelium. Morphological changes were confirmed by TEM observations.

We measured the transport rates of albumin and glucose through the bilayer and demonstrated that the increase in protein level is accompanied by more efficient uptake of both substrates. Our model can be employed to assess nephrotoxicity and other functions of the organ such as excretion of xenobiotics.

Presentation: Poster

10

A 3D bio-printed microphysiological system for modelling of human cerebrovascular disorders

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Despite tremendous research efforts by basic and clinical scientists, neurological diseases remain the main cause of disability worldwide. Neuronal cell death and degeneration have been the center of extensive research work in the field of neurological diseases; however, recent experimental shreds of evidence have led to a paradigm shift and more attention is being paid to the role of the brain vasculature. Defects in the blood-brain barrier (BBB) are associated with a variety of brain diseases, including brain cancer, stroke, and neurodegenerative conditions. Given the obvious discrepancies across species, manifested by the poor predictive power of animal models for human responses to drugs, the relevance of animal models to study neurological diseases is questionable. While conventional *in vitro* models are simply not conceived neither for capturing the tridimensional tissue architecture nor for recapitulating the highly dynamic events occurring at the BBB, microphysiological systems (MPSs) have recently emerged as an alternative solution. Here, we describe the development of a next-generation BBB-MPS obtained from the combined use of industrial 3D- and bio-printing methods to generate a robust and scalable model of the human BBB. This perfusable system recapitulates the endothelial-parenchymal interface of the BBB maintains high barrier function and allows for perfusion of human whole blood for the study of blood-vascular interaction in healthy and inflamed states of the BBB. Our results support the use of the BBB-MPS for a wide range of studies aimed at detecting potential drug toxicity and efficacy, as well as the basic processes underlying brain homeostasis and response to injury.

Presentation: Poster

11

Establishment of a human multi-organ-chip platform to replace animal transplant models for preclinical evaluation of Treg cell therapies

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The clinical development of advanced human cell therapies suffers from a lack of preclinical testing in laboratory animals. The informative value of such (humanized) animal trials are limited due to their phylogenetic distance to humans and, especially, their lack of a human immune system. Due to the histocompatibility mismatch between laboratory animals and the patient, challenges increase significantly once personalized regulatory T cell (Treg) therapy approaches for the prevention of transplant rejection are under evaluation. Adoptive transfer of Tregs is a promising therapeutic option to reshape intra-tissue immune imbalance in transplant patients. It aims at supporting long-term function of allografts by overcoming the challenge of undesired immune reaction by the recipient.

Here, we used the HUMIMIC® multi-organ-chip platform to establish a next-generation human *in vitro* assay for predictive preclinical testing of Treg products. The platform enables co-culture of various human organ models but lacks blood micro-capillary vessel structures covered with human endothelial cells.

For this purpose, we implemented a network of miniature vascularized channels in the organ compartments of the HUMIMIC® platform for two-organ co-culture exploring 3D printing tools and endothelial self-assembly processes. The organ models and endothelial cells were generated from iPSCs of two different individual HLA-tested healthy persons emulating the recipient and the donor background. Finally, we aimed to qualify a HUMIMIC® based next-generation transplant rejection assay to evaluate both, safety and efficacy of Treg products in a universal repeated dose long-term assay environment. Multi-organ-chip design and prototyping results are presented along with the results of iPSC-based differentiation of human endothelial cells, liver equivalents and kidney models for the establishment of the interconnected two-organ model. Furthermore, we present data on on-chip micro-vessel formation and co-culture over prolonged culture periods. Results will be discussed in the light of the assay potential to replace respective animal transplant models in use.

Presentation: Oral

12

Microphysiological systems for immune cell trafficking and capture

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Microfluidic technology has played a leading role to advance our understanding of fundamental biological processes including cell separation and isolation, next generation sequencing, and cell trafficking. Over the past five years our lab has applied the basic principles of microfluidics to control fluid shear and flow to create simple microphysiological systems to better understand: 1) how to capture and isolate rare immune cells from the peripheral circulation, and 2) the principles which guide and control immune cell (lymphocytes, monocytes, and neutrophils) trafficking in complex tissue microenvironments. For the former, we leverage the ability to coat surfaces with antigens that are recognized by rare populations of B lymphocytes in the peripheral circulation. We then control the shear force at the surface and can capture and isolate these rare cell populations. Understanding how these rare cell populations evolve over time following viral (e.g., SARS-Cov2, influenza) infection is central to understanding immunity following infection or immunization. For the latter, we are pursuing three projects. The first involves neutrophil trafficking into the cardiac muscle during COVID19-induced "cytokine storm", including counter-strategies that limit binding of neutrophils to the inflamed endothelium. The second involves modeling myeloid cell-directed immunosuppression in the tumor microenvironment, and how counter-strategies such as inhibiting STAT3 signaling, can enhance CAR-T cell trafficking and effector function. The third project describes small tumor cell-derived extracellular vesicle (sEV) transport (convection and binding to the extracellular matrix) and how the sEV can establish spatial gradients to guide monocyte migration in the tumor microenvironment. This talk will provide an overview of our major results from each of these projects.

Presentation: Oral

13

Body-on-a-chip: The potential to transform drug development

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A physiologically representative, multi-organ microphysiological systems (MPS) based on human tissues (also known as "human- on-a-chip"™) may be a transformative technology to improve the selection of drug candidates most likely to earn regulatory approval from clinical trials. Such microscale systems combine organized human tissues with the techniques of microfabrication based on PBPK (Physiologically Based Pharmacokinetic) models. They are "self-contained" in that they can operate independently and do not require external pumps as is the case with many other microphysiological systems. They are "low cost", in part, because of the simplicity and reliability of operation (e.g., pumpless systems). They maintain a ratio of fluid (blood surrogate) to cells that is more physiologic than in many other *in vitro* systems allowing the observation of the effects of not only drugs but their metabolites. While systems can be sampled to measure the concentrations of drugs, metabolites, or biomarkers, they also can be interrogated *in situ* for functional responses such as electrical activity (using microelectrode arrays) force generation using cantilevers), or integrity of barrier function. Operation up to 28 days has been achieved allowing observation of both acute and chronic responses with a totally defined medium that is serum free.

The key advantage of this approach is that we can determine in preclinical studies both the potential efficacy and toxicity of a drug candidate in a human surrogate. This advantage is dependent on having a complete multi-organ model with physiologically realistic relative organ volumes and flow rates of a blood surrogate. This design allows metabolites to form in one organ and circulate to other organs at physiologically relevant concentrations. We will discuss the origin of this concept, examples of its implementation, and speculate on its future.

Presentation: Oral

14

Human macrophage polarization determines bacterial persistence of *Staphylococcus aureus* in a liver-on-chip-based infection model

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The liver harbors 80% of tissue macrophages of the human body and represents a central organ in clearing pathogens from the bloodstream in the course of systemic infections. *Staphylococcus aureus* (*S. aureus*), a gram-positive bacterium representing an important human pathogen in community and hospital-acquired infections, has been reported to infect various organs ranging from asymptomatic colonization to severe infections and sepsis. Although considered an extracellular pathogen, *S. aureus* can invade and persist in professional phagocytes such as monocytes and macrophages. Its capability to persist and manipulate macrophages is considered a critical step to evade host antimicrobial reactions. We leveraged a recently established human liver-on-chip infection model and state-of-the-art algorithm-based image analysis to demonstrate that *S. aureus* specifically targets macrophages as an essential niche facilitating bacterial persistence and phenotype switching to small colony variants (SCVs). *In vitro*, M2 polarization was found to favor SCV-formation and was associated with increased intracellular bacterial loads in macrophages, increased cell death, and impaired recruitment of circulating monocytes to sites of infection. Our findings underline the potential of complex immunocompetent microphysiological systems to follow human bacterial infection mechanisms in a spatiotemporal manner to precisely dissect complex cellular interaction and to identify cellular targets for therapeutical intervention strategies.

Presentation: Poster

15

Cardiovascular MPS models for disease and drug discovery

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Our lab creates models for cardiovascular disease based on pluripotent stem cells (hPSCs). We use these for understanding disease mechanisms and cardiotoxic effects of drugs. Simple monolayer cultures of hPSC-cardiomyocytes or vascular cells are usually adequate for this purpose even though the cells are immature and fetal like. For example, by measuring contraction, action potential and calcium transients simultaneously in hPSC-cardiomyocytes, we demonstrated that we could predict the toxic effects of test drugs with almost 80% accuracy, compared with less than 70% in primary rabbit cardiomyocytes. For some purposes though, immature cells in 2D culture are insufficient to capture disease phenotypes. Examples that show the benefits of 3D culture of multicell type structures given: one model combines cardiomyocytes, cardiac fibroblasts, and cardiac endothelial cells in "microtissues" consisting of just 5000 cells. In these microtissues, cardiomyocytes show maturation including the formation of (post-natal) T-tubules. By replacing each of the cell types in the microtissues with a diseased variant, it was shown that cardiac fibroblasts carrying a PKP2 desmosomal gene mutation actually induce arrhythmia in microtissues in which the cardiomyocytes are normal and healthy. This indicates that fibroblasts in the heart can contribute to the phenotype in patients with arrhythmogenic cardiomyopathy. Another example is of a vascular disease in which the vascular cells behaved identically as the healthy isogenic controls in 2D vascular networks but the lumenized vessels were distinctly abnormal in 3D microfluidic "Organ-on-Chip" models. These more complex cell systems based on hPSCs are paving the way forward for a new generation of disease models for understanding disease mechanisms and drug discovery.

Presentation: Oral

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Development of a NAFLD model using human induced pluripotent stem cell and organ-on-chip technology

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Non-alcoholic fatty liver disorder (NAFLD) comprises a spectrum of increasingly harmful conditions ranging from steatosis to hepatocellular carcinoma. As the hepatic manifestation of the Metabolic Syndrome, NAFLD is closely associated with insulin resistance and diabetes. Therefore, steatosis is a major public health concern because of its increased prevalence worldwide and severe sequelae. In this work, we investigated the development of a relevant NAFLD model using hepatocytes-like cells (HLC) derived from human induced pluripotent stem cells (hiPSCs) and organ-on-chip technology.

Our protocol consisting of a step-by-step differentiation process from definitive endoderm (S1) to hepatic specification (S2), followed by a hepatic progenitor step (S3) and finally reaching HLC (S4). hiPSCs (ChiPSC22, TakaraBio) were cultivated on static condition in standard Petri dishes until S3, then detached and transferred in biochips for dynamic S4-maturation and exposition. Steatosis was induced by free fatty acid exposures (oleic acid). HLC differentiation and functionality was assessed by staining or/and measuring the levels of albumin, urea and CYP3A4. HLC mRNA levels of hepatic differentiation genes and steatosis markers were also investigated. Finally, HLC steatosis state was evaluated by staining and measuring cytoplasmic lipid droplets.

Our results shown cells with typical cuboidal hepatic phenotypes at differentiation end point. Moreover, albumin and urea detection demonstrated hepatic profile and basal functionality of HLC. Finally, lipid droplets presence was confirmed in cells exposed with fatty acids whereas no lipid accumulation was observed in controls.

The present study successfully allowed to propose a liver organ-on-chip model based on Takara Bio hiPSCs. The hepatic differentiation toward HLC was confirmed and the steatosis assays revealed the potential of our protocol to generate a liver-like disease model. To go further in our investigations, the kinetics of the disease's development will be investigated in order to track the biomarkers apparition and the disorder key time points.

Presentation: Poster

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Engineering spatially organized organs-on-chips

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The function of human organs is typically derived from intricate 3D patterns of specialized cells, which are modularly assembled to perform integrated vital functions. Unifying different levels of complexity into *in vitro* models is an ongoing challenge, which demands intricate interdisciplinary crosstalk that combines efforts from (micro-tissue) engineering, material chemistry, and life sciences. This presentation will focus on highlighting examples of spatially organized, dynamic, microenvironments within organ-on-chip (OoC) platforms, as key drivers of physiological function and inter-tissue communication. Specifically, I will present: i) OoC microdevices designed to incorporate 3D instructive elements that mimic structural and functional complexity, more specifically, describing a 3D physiologically inspired alveoli-on-chip model; ii) multi-OoC approaches, intended to replicate human disease states involving several tissues, namely a joint-on-chip model to study osteoarthritis, which also incorporates micro-actuators to generate a dynamic and realistic cell culture microenvironment; and, iii) overview of a standardization strategy that will render OoCs compatible with parallelization and integration, towards a more high-content/high-throughput screening approach via incorporation of sensing and automation. Moreover, by integrating advanced bioprinting technologies with OoC, a higher degree of complexity and functionality can be added to these model systems, offering unique opportunities to create highly spatially controlled engineered devices, with cell-level resolution. The raising of technologies geared to further improve OoC will lead to more representative models, expected to provide long awaited mechanistic insights over (complex) diseases and increase the predictive accuracy of drug screening.

Presentation: Oral

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Establishing a skin-lung-lymph node co-culture in an organ-on-a-chip platform to emulate atopic diseases *ex vivo*

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Background: Atopic diseases are allergy-associated inflammatory conditions that are T helper type 2 (Th2) cell-mediated, examples include atopic dermatitis (AD), allergic rhinitis (AR), and allergic asthma (AA). AD often develops in early childhood and signals the potential subsequent onset of food allergy, AA and AR. To date, the mechanisms of it remains unclear. Further understanding of AD and the correlation between the onset of AD and the bronchial epithelial conditions will promote the development of more effective treatment for AD and ultimately, preventative approaches for other atopic diseases.

Methods: In vitro skin (healthy and diseased) and lung models were generated as described elsewhere. The skin-lung co-culture was established in a microfluidic multi-organ-on-a-chip (OOC) platform to emulate "skin-lung cross-talk". A novel anti-inflammatory compound was applied to the diseased co-culture with perfusion of activated $CD4^+$ T cells to test drug efficacy. A novel lymph node (LN) model was established with fibroblastic reticular cells and $CD4^+$ T cells. Characterizations were performed with ELISA, cytotoxicity assay, histological and immunofluorescent staining to all models, respectively.

Results: The skin-lung models cultured in the microfluidic OOC platform demonstrated physiological characteristics as of *in vivo*, including morphology, histology, and protein expressions. The compound applied to the co-culture blocked systemic Th2 cytokines expression via inhibiting thymic stromal lymphopoietin signaling pathway and showed non-toxic at its effective concentrations on the OOC platform. The developed LN models demonstrated physiological characteristics including connective tissue framework and homing of $CD4^+$ T cell as of *in vivo* lymphoid organs. Similar to LN *in vivo*, migration of the already-homed $CD4^+$ T cell upon chemokine stimulation was confirmed in our model.

Conclusion: We have developed an immunocompetent skin-lung co-culture that closely mimic the characteristics of atopic diseases *in vivo* and was suitable for the prediction of drug efficacy of a novel anti-inflammatory small molecule.

Presentation: Poster

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Kidney-on-a-chip – Integrating glomerular filtration and tubular reabsorption models

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

In this study, we generate an autologous kidney-on-a-chip that encompasses a glomerular and a tubular model. Induced pluripotent stem (iPS) cell-derived podocytes and tubular cells are seeded into the HUMIMIC Chip4, which enables the long-term co-cultivation of the renal model with up to three additional organ equivalents with a defined fluid flow and shear stress. The final maturation of the iPS cell-derived podocytes and tubular cells occurs within the Multi-Organ-Chip, thereby allowing the cells to form a barrier that enables glomerular filtration and tubular reabsorption. After the renal cells' final maturation, the co-culture can be maintained for at least 14 days. The kidney-on-a-chip exhibits a stable metabolism, a cellular barrier that prevents albumin from entering the excretory circuit, and the cells demonstrate a steady expression of key podocyte and tubular markers.

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

Presentation: Poster

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Neural Rosette Arrays™ for quantitative high-throughput screening of human developmental neurotoxicity and teratogenicity

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Human pluripotent stem cell (hPSC)-derived neural organoids, which recapitulate central nervous system (CNS) development, are powerful tools for investigating human neurophysiology and disease. However, inconsistencies in emergence during derivation limit their reproducibility and hinder scalability for commercial application. The Rosette Array™ platform (doi:10.7554/eLife.37549) presented here standardizes neural organoid morphogenesis by exerting microscale spatial control over cues normally present in the developing human embryo. This enables efficient, reproducible, and high-throughput derivation and analysis of hPSC-derived neural organoids that mimic the earliest stages of CNS development, i.e., neural tube formation. Further, this platform provides a technological basis from which to expand more complex micro-physiological CNS models for commercial toxicology and drug discovery applications.

Here, we present validation of the Rosette Array™ for use as a developmental neurotoxicity (DNT) screening tool with focus on identifying teratogenic effects, i.e., assessing neural tube defect (NTD) risk – the second most prevalent class of birth defect (manuscript in review). Through screening a library of pharmaceutical, agrochemical, and industrial compounds, many of which are known to cause NTDs, we have established a high-throughput pipeline to identify compounds that perturb microarrayed rosette tissue formation and provide insights into their mode of action. Specific DNT results for select compounds will be presented, highlighting both previously unreported NTD risk assessments and region-specific differences in drug response, which is a unique aspect of the Rosette Array™ platform afforded by the ability to derive organoids corresponding to diverse CNS regions. Additionally, we will summarize our automated image analysis pipeline for detecting pathological disruption to organoid morphogenesis. In summary, the Rosette Array™ platform significantly streamlines and increases the human relevance of *in vitro* DNT screening for chemical and drug discovery pipelines.

Authors declare a conflict of interest due to holding equity shares in Neurosetta LLC, which aims to commercialize Rosette Array™ technology.

Presentation: Oral

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Fully defined and standardized human macrophages from iPSC as a new tool for next generation and high throughput drug efficacy and safety evaluation

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In the field of drug development, screening pharmaceuticals for safety and efficacy is a major challenge. This is particularly essential for parenteral products ranging from chemical drugs to cell-based therapeutics. Given their role as first line responders to drugs and other molecules, cells of the human innate immune system (e.g., macrophages) are highly needed for industrial use. In fact, some of the safety- and screening-platforms rely on primary human blood cells and their integration into Microphysiological system (MPS), which lack the contribution of immune cells, is currently explored. Of note, blood from human donors show high donor to donor variability in addition to the allogeneic reaction profile, both factors that minimize regulatory acceptance and the establishment of complex MPS. As an alternative, we here introduce the generation of fully standardized macrophages from human induced pluripotent stem cells (iPSC) in scalable quantities using modern differentiation methods. iPSC and thereof derived macrophages (iMonoMac) are fully defined and can be produced in reproducible quality and quantity from the same donor. As a first assessment, we used iMonoMac as a new cell source in the evaluation of parenteral drugs contamination with pyrogens (known as Monocyte-Activation-Test (MAT)). Using industry compatible differentiation platforms, iMonoMac showed typical macrophage like morphology and stained positive for CD14, CD163, CD45, CD11b, and TLR2, TLR5, TLR4. Furthermore, iMonoMac were more sensitive compared to PBMC or MonoMac6 cell line when stimulated with a broad range of pyrogens including endotoxin, non-endotoxin-based, and process related pyrogens at a high dynamic range. Given the success of iPSC in regenerative medicine, clearly the generation of different immune cells from the same iPSC line would accelerate establishing complex MPS in the near future. In summary, our new technology on the continuous production of immune cells would facilitate the development of new assays to screen and evaluate pharmaceuticals.

Presentation: Poster

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Open platform technology for organs-on-chips

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Lack of standardization, automation and inter-chip incompatibility is limiting innovation and implementation of organs-on-chips. Organ-on-chip users should be able to freely combine organ-chips, sensors and actuators to obtain a model that specifically answers their questions of interest. To achieve this, standards for organ-on-chip interfacing are needed. However, their implementation is hampered by the existence of multiple commercial platforms that all have their own design philosophy.

To overcome this challenge, organ-on-chip platforms are needed that are both "open" and "modular". An "open" platform facilitates and invites active contributions from many stakeholders. A 'modular' platform enables interactions between components on different levels through standardized interfaces.

We are developing a platform to provide infrastructure for automated microfluidic chip control, open for academic and commercial chip developers: the Translational Organ-on-Chip Platform (TOP). The platform relies on microfluidic building blocks (organs-on-chips, sensors, reservoirs, etc.) that interface with each other via a fluidic circuit board. The fluidic circuit board routes liquids from one building block to another and offers a standard form-factor with the footprint of a microwell plate.

We have used TOP in multiple projects and have demonstrated how it can enable interaction between components from different developers (e.g., by connecting commercial flow sensors to in-house heart-on-chip devices). Moreover, we have shown that the platform can be used for automation of complex microfluidic workflows (e.g., by connecting a microfluidic mixing device with a multiplexed cell culture chip).

In this talk, the concept of TOP as an open platform for organs-on-chips is defined from a technical perspective. Moreover, we discuss the importance of involving stakeholders in the development, manufacturing, and application of such an open platform. Several examples of current use cases of TOP are provided. Finally, we provide an outlook on the role that open platform technology will play in working towards standards for organs-on-chips.

Presentation: Oral

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Establishment of a 3D multi-organ system to study chronic lymphocytic leukemia (CLL) dissemination *in vitro*

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CLL is a dynamic neoplasia characterized by the accumulation of mature B cells interacting with a supportive tissue microenvironment. CLL cells do not survive long when cultured *in vitro* in 2D cell cultures likely due to the lack of endogenous stimuli and of the spatial organization complexity present *in vivo*.

An innovative solution could be the 3D modelling of the disease *in vitro*. Our group is focusing on dissecting CLL and stromal cells behavior in 3D systems, integrating advanced biomaterials and millifluidic systems aiming to obtain an *in vitro* model of CLL. Our aim is to create a perfused, multiorgan model constituted by interconnected Bone Marrow (BM) and Lymph Node (LN), in which to circulate CLL cells in order to obtain a more predictive model to investigate CLL dissemination and behavior concomitantly with the use of target therapies.

We take advantage of a gelatin-based scaffold as matrix in which to seed cells to be maintained in static and dynamic conditions by means of a peristaltic pump (IVTech). We optimized seeding and culture protocols and we are working on the characterization of our 3D models comparing static and dynamic conditions. In particular we observed a better organization of the stroma in the samples under fluid flux, highlighting a better structured vascular network also expressing endothelial markers like CD31, EMCN and VEGFRIII. Preliminary results on CLL cells circulation showed a higher propensity for homing in the LN and BM-like structure compared to a 3D simplified culture mimicking blood vessel.

We here show the feasibility to obtain 3D lymphoid tissues models that mirror the corresponding compartments *in vivo*. The next step will be to recirculate CLL cells between the different compartments in a multi-organ system and analyze possible modifications in terms of tissue architecture, gene expression and production of soluble factors.

Presentation: Poster

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Longitudinal imaging of non-small cell lung carcinoma organoids via label-free multimodal multiphoton microscopy

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The DNA damage response (DDR) is a key metric for evaluating potential cancer therapeutics, many of which target DNA or associated processes (e.g., cisplatin: DNA crosslinker, methotrexate: prevents DNA synthesis, Niraparib: inhibits DNA repair). Current techniques to evaluate DDR rely on immunostaining for pH2AX, which serves as an indicator of DNA double-strand break formation. While pH2AX immunostaining is capable of providing a snapshot of DDR in fixed samples, this methodology is disadvantageous because: 1) temporal monitoring of the DNA damage response is costly and labor intensive; and 2) selected time points are arbitrary, resulting in a need to redo assaying if DDR occurs before or after selected timepoints. To improve the resolution of DDR evaluation and reduce its costs, label-free multimodal multiphoton techniques such as simultaneous label-free autofluorescence multiharmonic (SLAM) imaging and fluorescence lifetime imaging microscopy (FLIM) are proposed to monitor DDR in live samples. Ongoing investigation using SLAM and FLIM on human non-small cell lung cancer (NSCLC) organoids have demonstrated the capability of label-free multiphoton microscopy to interrogate cell features such as morphology, heterogeneity, and metabolic molecular profiles, without labeling. We aim to investigate whether these label-free cell features can be used to monitor DDR in NSCLC organoids by using pH2AX immunostaining as the ground truth indicator of DDR. The result of this study will be to identify and validate features indicative of DDR using label-free multiphoton microscopy of live cultures of NSCLC organoids to enable real-time monitoring of DDR.

Presentation: Poster

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Human-based neuromuscular system for personalized ALS modeling and drug testing

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of muscle innervation and motoneuron (MN) degradation. Despite the heterogeneity in pathogenesis, one early critical hallmark is shared by all ALS cases which is the loss of the neuromuscular junction (NMJ). We have developed a human-based functional NMJ model for ALS by utilizing patient-derived induced pluripotent stem cells (ALS-iPSCs). The human-based serum-free co-culture system supporting functional NMJ formation was developed and adapted to a dual-chamber system through integration with BioMEMs technology, in which NMJ functionality can be interrogated quantitatively and drug effects can be evaluated in a dose-dependent manner separately in each chamber. MNs differentiated from ALS-iPSCs (ALS-MNs) were characterized for ALS-specific phenotypes and integrated into this NMJ platform. Functional analysis revealed significant NMJ deficits as characterized by a set of clinically relevant parameters including NMJ quantity, fidelity and fatigue index. Treatment of the ALS-NMJs with the Deana protocol demonstrated effectiveness in all the tested ALS mutant lines with some mutant-specific variations. Similarly, skeletal muscle cells (SKM) differentiated from ALS-iPSCs (ALS-SKM) demonstrated reduced differentiation efficiency, severe structural atrophy, decreased contractile force and fidelity upon stimulation, as well as deficits in mitochondria function and cell metabolism. After being integrated into the NMJ platform, the chambers containing ALS-SKM demonstrated deficits in similar parameters as those carrying ALS-MNs, but with a relatively more pronounced severity. Combination of ALS-MNs and ALS-SKM in the NMJ system demonstrated a potentiated phenotype more severe than those having mutations in either cell type alone. These findings highlighted the active and significant role of SKM in ALS pathogenesis and its potential as a therapeutic target. These functional ALS NMJ models recapitulate clinically relevant pathological phenotypes thus provide a platform for ALS etiological investigation and patient stratification for drug testing.

Presentation: Poster

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3D-engineered kidney tissue chips recapitulate species-specific toxicological profiles in response to polymyxin B

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Failures of compounds contributing to the high attrition rate of drugs in clinical trials can largely be attributed to poor translational predictability of human physiological outcomes during preclinical screening of compounds *in vitro*, and in animal models. Advances in the organ-on-chip (OOC) field have demonstrated its potential as a powerful predictive technology for assessing drug-induced kidney injury. In the current study, we assessed whether distinct species-specific toxicological profiles can be detected in a kidney OOC model in response to polymyxin b (PMB), an antibiotic known for its dose-limiting nephrotoxic effects in humans and animal models. Renal proximal tubule epithelial cells of human, dog, and rat were grown in standard 2D culture plates or 3D-engineered into continuously perfused tubules within microfluidic chips. Both 2D and 3D cultures were subjected to PMB at concentrations ranging from 200-2000 µM continuously for 48 h with media sample collection at different timepoints throughout the experiments. Cytotoxicity was assessed by measuring levels of ATP in media via bioluminescence, and cell viability was determined through staining with calcein-AM and ethidium homodimer-1 for detection of live and dead cells respectively. Levels of the protein KIM-1 were measured via colorimetric ELISA immunoassays to assess differences in its efficacy as a marker for early injury between species. The results of our study show that the OOC toxicological profiles of each species resemble that of *in vivo* physiology observed in animal studies and the clinic, in contrast to 2D, where toxicity continued to rise to unphysiologically high levels. This study demonstrates the OOC model's ability to detect interspecies sensitivity differences to PMB, reflected by unique species-specific toxicological signatures in response to PMB. Kidney OOC models appear to serve as useful tools for predicting drug candidate safety in clinical trials and can be used to promote higher efficiency in animal studies.

Presentation: Poster

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Myelination and node of Ranvier formation in a human motoneuron-Schwann cell serum-free coculture

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Myelination and subsequent 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. We report a human-human myelination model using primary Schwann cells (SCs) and human-induced pluripotent stem-cell-derived motoneurons utilizing a serum-free medium supplemented with ascorbate to induce myelination, where 41.6% of SCs expressed the master transcription factor for myelination, early growth response protein 2. After 30 days in coculture, myelin segments were visualized using immunocytochemistry for myelin basic protein surrounding neurofilament-stained motor neuron axons, which was confirmed via 3D confocal Raman microscopy, a viable alternative for transmission electron microscopy analysis. The myelination efficiency was 65%, and clusters of voltage-gated sodium channels and the paranodal protein contactin-associated protein 1 indicated node of Ranvier formation. This model has applications to study remyelination and demyelinating diseases, including Charcot-Marie Tooth disorder, Guillain-Barré syndrome, and anti-myelin-associated glycoprotein peripheral neuropathy. Taking the work a step further, primary human Schwann cells were replaced by iPSC derived human Schwann cells, allowing differentiation of disease state Schwann cells, in order to study myelination in diseases such as Charcot-Marie Tooth, as well as Amyotrophic lateral sclerosis, where Schwann cells have recently been proposed as contributors of disease pathology. Establishment of this iPSC-based disease model also allows a platform to test therapeutics that could potentially ameliorate these diseases, with the potential for greater translatability to human patients than current animal models allow. This model could also be adapted onto a microelectrode array system to allow measurement of conduction velocity, providing a functional readout in addition to the collected biomarker information which would be extremely useful when it comes to testing of drugs to treat diseases where the myelination and action potential conduction is impaired.

Presentation: Poster

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Molecular characterization of a 3D bioprinted chronic lymphocytic leukemia (CLL) *in vitro* model for drug screening

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The traditional *in vitro* culture systems cannot fully reproduce the physiological features and complexity of human diseases. In the case of chronic lymphocytic leukemia (CLL), the short survival of primary leukemic cells cultured in 2D, makes it difficult to fully characterize their molecular and biological phenotype.

We established a long-term culture model for CLL primary leukemic cells and demonstrated that the bioprinting process does not affect cell viability, resulting in an increased survival (up to 28 days) as compared to 2D cultures. RNA Seq analyses highlighted molecular changes following the leukemic cells adaptation to the 3D culture. (Sbrana et al., 2021).

We aim at investigating the role of a 3D reconstructed environment in the response to novel therapeutics agents currently used in clinic for CLL, such as BCL-2 inhibitors, by exploiting dynamic systems to closely mimic the *in vivo* condition and to pave the way for advanced platforms for personalized drug screening purposes.

Among them we are using the MIVO® bioreactor and a brand-new device fully developed by our laboratory through 3D printing technology, adding to the system a vascularized environment. In those settings we will assess CLL cells extravasation, homing, and response to drugs. We are comparing 2D vs 3D and static vs dynamic settings to elucidate the differences in term of response to drugs.

The use of 3D bioprinting technology enables a deep characterization of CLL cells *in vitro* thanks to a 3D structure that improves the cellular well-being over time and induces a functional adaptation resembling the *in vivo* tissue condition. Furthermore, its combination with millifluidic culture systems open up new ways for studying vascularized constructs in a physiologically relevant context.

Presentation: Poster

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In vitro three-dimensional tissues on microdevices for modeling neuromuscular junction-related diseases and testing drug efficacy

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Skeletal muscle contraction is induced by the signals from the motor neuron through the neuromuscular junction (NMJ). Since dysfunction of the NMJ causes various neuromuscular diseases, *in vitro* system for modeling NMJ is required to improve our mechanistic knowledge on the NMJ-related diseases and develop effective treatments. Previously, we have developed engineered skeletal muscle tissues on the microdevices for measuring their contractile force and applied them for modeling skeletal muscle atrophy and testing drug efficacy (Shimizu et al., 2017; Nagashima et al., 2020). In the present study, we aimed to construct *in vitro* three-dimensional tissues in which human iPSCs co-differentiated into motor neurons and skeletal muscle cells and to evaluate the function of the constructed neuromuscular tissues using contractility as an index. Human iPSCs which transiently express MYOD1 by doxycycline treatment were used in this study. The iPSCs differentiated into skeletal muscle cells on a dish were harvested and used for constructing tissues on the microdevice. The tissue on the device was kept cultured in the medium for skeletal muscle cells and subsequently the medium was changed to the medium for motor neurons induction. We confirmed by real-time PCR and immunofluorescence staining that the myotubes and motor neurons were co-existed in the tissues. Since the contraction of the tissues was induced by the treatment of glutamate and the contraction was inhibited by vecuronium bromide, it was suggested that the functional NMJ was formed in the tissues. The developed contractile tissues would be used for modeling NMJ-related diseases and testing drug efficacy.

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Presentation: Poster

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A microphysiological system to model the endothelial-epithelial interface

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Microphysiological Systems (MPSs) or organs-on-chips, are microfluidic devices used to model human physiology *in vitro*. Their purpose is to capture the relevant biology of a physiological phenomenon in order to better study it. Polydimethylsiloxane (PDMS) is the most widely used material for organs-on-chips due to its established fabrication methods and biocompatibility properties. However, non-specific binding of small molecules limits PDMS for drug screening applications. Here, we designed a novel MPS that mimics the architecture of the endothelial-epithelial interface (EEI). The EEI is pervasive throughout the body and is heavily implicated in proper vascular function. To reconstruct this biology, we designed a membrane-based chip that features endothelial cells on the underside of the membrane exposed to mechanical shear from the path of media flow, and epithelial cells on the opposite side of the membrane protected from flow as they are *in vivo*. We used a liver model with a hepatic progenitor cell line and human umbilical vein endothelial cells to assess the biological efficacy of the MPS. We computationally modeled the physics that govern the function of perfusion through the MPS. Empirically, efficacy was measured by comparing differentiation of the hepatic progenitor cells between the MPS and 2D culture conditions. We demonstrated that the MPS significantly improved hepatocyte differentiation, increased extracellular protein transport, and raised hepatocyte sensitivity to drug treatment. Our results signify that perfusion has a profound effect on proper hepatocyte function. The capacity of perfusion to increase nutrient transport incites opportunities for future study of multi-organ interactions.

Presentation: Poster

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A robust, high-throughput electrophysiology platform for drug screening in a peripheral nerve-on-a-chip microphysiological system

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Microphysiological systems (MPS) have the potential to accelerate drug development by screening compounds using systems that better mimic *in vivo* physiology compared to existing cell culture or animal models. The potential for lower cost, higher throughput compound screening with more physiologically relevant data has gained increasing attention from the pharmaceutical industry in the hope that MPS will reduce attrition rates. AxoSim has developed an innovative MPS, the NerveSim® platform, for screening compounds using an embedded electrode array (EEA) to record compound action potentials (CAPs) from neuronal cultures.

The NerveSim® EEA platform is a custom 24-well tissue culture plate with an integrated circuit board to allow high-throughput electrophysiological recordings. Each well contains a cell-restrictive mold that is filled with a grow-permissive inner gel forming a 9 mm channel. Neuronal spheroids are placed at one end of the channel and axonal growth is guided through the inner gel to create a 3D nerve-like bundle. Below the inner gel are a series of 10 widely spaced microelectrodes that can be used for both recording and stimulating the nearby axons.

Electrophysiological recordings are accomplished using a custom headstage that connects to the EEA plates and allows recording of 12 wells simultaneously inside a standard tissue culture incubator. In a typical experiment, 12 EEA wells are stimulated in parallel at multiple distal sites with a stimulation current ramp while recording the CAPs at the spheroid and axons. Automated stimulation via scripting minimizes operator error while maximizing efficiency, generating highly reproducible electrophysiological data. From these data, clinically relevant electrophysiological metrics such as the conduction velocity, response amplitude, and threshold stimulus strength are measured and compared against vehicular controls. The non-invasive nature of the recordings allows measurement of the same cultures at multiple timepoints, before and after drug dosing, to track how electrophysiology changes over time.

Presentation: Poster

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A novel tissue bioreactor for retinal organoid microenvironmental control

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In vitro culture systems generally apply homogeneous stimuli and rely on intercellular signaling to guide growth of tissues. However, to derive complex tissue structures such as the human retina, a gradation of certain stimuli is required. The inner retina resides in a hypoxic environment (2% O_2) adjacent to the vitreous cavity. From there, oxygenation levels rapidly increase towards the outer retina (18% O_2) at the choroid. Here we developed a novel tissue bioreactor allowing the maturation of inner and outer retinal cell phenotypes within an O_2 gradient.

The bioreactor is assembled from a 75x25x3 mm acrylic slide, a PFA film, a cover glass, and double-sided adhesives, which were adjusted with computer numerical control milling and laser cutting. The 60 culture wells of 2 mm in diameter and 0.7 mm high each hold one retinal organoid. A nitrogen (N_2) tank provides the bioreactor with 5 mL/min N_2 gas and a dual syringe pump creates a 5 µL/min continuous flow of culture medium though the bioreactor. Gas diffusion through the gas permeable PFA membrane and culture medium was predicted using computational modeling software for atmosphere (20.9% O_2) and incubator (18.6% O_2) conditions. O_2 concentration measurements were performed with O_2 sensors along the z-axis in 50 µm steps in atmospheric conditions.

The gas diffusion throughout the culture medium resulted in an O_2 concentration gradient along the z-axis. The computational predictions in atmospheric conditions are in accordance with the measurements around the retinal organoid location in the bioreactor.

In conclusion, this open-well bioreactor is easily accessible for downstream analysis, establishes a steep O_2 gradient and allows high-throughput retinal organoid culture. It will help retinal organoids mature into the complex structure to use them for disease modeling and drug testing.

Presentation: Oral

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Multicellular spheroid culture using egg white as an economically viable platform for application in microphysiological systems

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Multicellular spheroids are probably the most classic 3-D culture approach in which cell-cell and cell-ECM interactions are key factors that have been preserved to provide complex biological and mechanical signals for the maintenance of native cell biology in cultured cells. Consequently, this 3-D spheroid model mimics the *in vivo* environment better than 2-D cell-based models to deliver better reliability for pharmaco-toxicology applications. Enhanced cell surface receptor-mediated cell adhesion, differential gene expression, dynamic micro-environment, and genetically more representative to organotypic explants are key advantages that the spheroids offer compared to monolayer cells.

Various materials and methodologies have progressively been developed for the design of an efficient multicellular spheroid model. In this study, extracted egg white (EW) from hens (*Gallus gallus domesticus*; White Leghorn breed) was used as a platform for spheroid culture, as it is an easy-to-prepare and a cost-effective platform to serve as an alternative to polysaccharides, cellulose, and synthetic scaffolds. Physicochemical characterization in our earlier research indicates that EW possesses nano-to micron-level structures and favorable viscoelasticity properties that support the formation of spheroids. Besides, protein-based bioactive substances present in the EW promote cell growth without the need for additional ECM components. These parameters were identified as a benefit of using EW as an effective platform for the culture of 3-D spheroids.

Here we summarize a standardized, reproducible protocol for EW extraction, 3-D spheroid cell culture, routine spheroid monitoring, and a convenient method for MTT testing to measure both the rate of proliferation and the viability of cells in spheroids compared to monolayer cell culture. Our main intention here is to provide a comprehensive overview of the procedures with technical pitfalls and troubleshooting that have arisen when developing spheroids in the context of microphysiological systems.

Presentation: Poster

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Characterizing the reproducibility of a liver microphysiological system for assaying drug toxicity, metabolism, and accumulation – Joint study by an MPS developer and a regulator

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Liver microphysiological systems (MPS) are advanced cell culture models that can model tissue level functionality of the human liver and are a promising tool for predicting hepatic drug effects. Yet, after a decade since their introduction, liver MPS are not routinely used in drug development, partly due to lack of criteria for ensuring reproducibility of results.

We characterized the feasibility of a liver MPS to yield reproducible data for experiments assaying drug-induced liver injury (DILI), phase I and phase II metabolism, and intracellular drug accumulation. The ability of the liver MPS to reproduce hepatotoxic effects was assessed using trovafloxacin, which is associated with a high risk of DILI. Trovafloxacin treatment of liver microtissues in the MPS increased lactate dehydrogenase (LDH) release and reduced cytochrome P450-3A4 (CYP3A4) activity. These observations were made across two test sites and with multiple batches of Kupffer cells.

Culturing equivalent hepatocytes in the MPS, spheroids, and sandwich cultures, differences between culture formats were detected in CYP3A4 activity and albumin production. Cells in all culture formats exhibited different sensitivities to hepatotoxicant exposure. Hepatocytes in the liver MPS were more functionally stable than those of other culture platforms, as CYP3A4 activity and albumin secretion remained prominent for greater than 18 days in culture, whereas functional decline occurred earlier in spheroids (12 days) and sandwich cultures (7 days).

The MPS was demonstrated to be suitable for drug metabolism and pharmacology studies, where troglitazone metabolites, diclofenac clearance, and intracellular accumulation of chloroquine were quantified. Finally, the use of LDH and CYP3A4 assays were implemented throughout the liver MPS cultures as quality control metrics to ensure reproducibility of data between individual cultures. Overall results indicated that the liver MPS can be used reproducibly in drug evaluation applications and the study outcomes led to general considerations and recommendations for using liver MPS.

Presentation: Oral

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Why two organs are better than one: A gut-liver microphysiological system that mimics human oral and intravenous dosing regimens for improved prediction of oral bioavailability

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Accurately predicting the bioavailability of orally administered medicines in humans during pre-clinical development is crucial as it forms the basis for setting safe and efficacious doses in the clinic. Animal models dominate the study of human bioavailability, but their prediction is known to be poor ($R^2 = 0.34$; Musther et al., 2014) and best reserved as a qualitative estimate, i.e., low vs high. The limitation of current *in vitro* models, both static and single organ microphysiological systems (MPS) is a lack of complexity. This is because bioavailability is dependent on the functions of two organs: permeability of drugs through the intestinal wall and hepatic first pass metabolism in the liver.

To solve this problem, we introduce a multi-organ MPS that consists of six wells, each with two compartments, a Transwell® (gut epithelium) compartment and 3D liver compartment, which are fluidically linked. The gut-liver MPS is comprised of intestinal epithelial cells cultured on a permeable Transwell insert (mix of Caco-2 and HT-29 cells) and primary human hepatocytes, which form 3D microtissues on a collagen-coated scaffold in the liver compartment. For predicting bioavailability, we replicate the basic methodology of *in vivo* studies by simulating an oral and intravenous dosing regimen. Predictions are made on four drugs with known human and animal bioavailability, including three that are low clearance.

We show improved correlation with the gut-liver MPS ($R^2 = 0.9$, $n = 4$) to known human oral bioavailability compared to data from animal models ($R^2 = 0.28$, $n = 4$). This application demonstrates the power of a multi-organ MPS to predict parameters such as bioavailability, where the distinct functions of two organs are needed.

Reference

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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 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 $ROR1^+$ breast cancer spheroids (MDA-MB-231 ffluc-GFP), embedded in a dextran-based hydrogel. Fluorescently tagged ROR1-targeting CAR-T cells (or control untransduced T cells from the same donor) with an equal ratio of $CD4^+$ and $CD8^+$ T cell population were constantly perfused (20 µL/h) through media channel lined with primary microvascular endothelial cells that is located above the tumor chambers. The antitumor response was assessed via multimodal quantitative imaging analysis and the monitoring of inflammatory cytokine release profile (IL-2, IL-6, IL-10, granzyme B, IFN-γ, and TNF-α) during 8 days of culture.

During the first 15 h upon administration, CAR-T cells extravasated the endothelium faster than the control T cells. After 24 h, both control T cells and CAR-T cells infiltrated the tumor spheroids. However, control T cells were pushed to the periphery of the continuously growing tumor spheroids, whereas CAR-T cells stayed within the non-growing tumor spheroids during 8 days of culture. Additionally, cytokine levels typically observed during CAR-T cell-associated CRS were recapitulated, which were significantly higher than in the control T cell condition at almost all measurement timepoints.

The ability to recapitulate the efficacy and safety aspect of CAR-T cell therapy in this model will enable a better understanding of the TME role in hampering the efficacy in solid tumors, as well as provide new insights into the pathogenesis of CRS in a controllable and fully human setting.

Presentation: Poster

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A method to generate perfusable cylindrical physiologic-like vascular channels within organs-on-chip

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The recapitulation of 3-dimensional (3D) architecture of native organs, including their vasculature, is paramount for the generation of functional *in vitro* models. Advanced Organs-on-Chip (OoC) platforms have been recently developed, able to provide biochemical and physical stimulations to enhance tissue-specific functionality. However, these models present either separating membranes or scaffolds between the parenchyma and endothelial cells, introducing physical barriers absent in the native milieu. Moreover, they fail to recapitulate the intrinsic circularity of vascular channels, which is known to promote barrier functionality and cytoskeletal alignment of endothelial cells (Polacheck, 2019). To address these needs, we developed a method to generate on-chip a perfusable cylindrical endothelial channel embedded in a 3D construct without any physical separation. The device comprises two layers, namely (i) a culture chamber composed by a central channel for 3D cell-laden hydrogel confinement, flanked by two lateral channels for medium replenishment and (ii) a tank layer for cylindrical lumen obtainment. We implemented the method in a novel Liver-on-Chip platform: hepatocytes (e.g., HepG2, PHH, iPSC-derived hepatocytes) were embedded in a hydrogel, generating a 3D construct surrounding an inner vascular channel, lined by endothelial cells (e.g., HUVECs, LSECs). Liver functionality was assessed through albumin and enzymatic assays, immunofluorescence staining as well as RT-PCR of peculiar hepatic biomarkers. Endothelial cells expressed typical endothelial adherens-junction (VE-cadherin, CD31) and tight junction (ZO-1), with a low permeability (confirmed by 4kDa FITC-Dextran diffusion test). The 3D vascularized liver model can be thus used as a potent tool for drug screening applications, as well as to study the behavior of the immune component flowing in the vasculature in phato-physiological conditions. Additionally, the proposed platform is compatible with the uBeat® technology (Marsano, 2016), providing microtissues with a controlled mechanical stimulation thus resulting suitable with other applications (e.g., heart, gut) in the OoC field.

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Presentation: Poster

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A valve-based microfluidic platform for controlled diffusion of liver-metabolized drugs

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Owing to microfabrication techniques, cell patterning has been widely shown to play a major role in modelling physiological environments (Pitaval et al., 2010). For instance, liver models based on micropatterned co-cultures (MPCCs) can maintain stable functions for weeks *in vitro* (Khetani and Bhatia, 2008). Here we integrate the MPCC model in a valve-based Multiorgan-on-Chip platform encompassing liver-tumor compartmentalized cultures (Ferrari et al., 2021) and we show its application in studying the effects of liver-metabolized Tegafur on tumor tissue. The platform layout encompasses two culture chambers for liver and tumor cultures, separated by normally closed PDMS valves. These valves can be opened through an overlaying vacuum-activated control layer to impose communication between compartments. To evaluate mass diffusion between adjacent compartments, low molecular weight (4 kDa) FITC-dextran was injected in the liver chamber and its concentration was monitored in the tumor chamber. MPCCs of HepG2 and 3T3 fibroblasts were seeded in the liver chamber whereas HCT-116 cancer cells were cultured in the tumor chamber of the device, and medium was changed every 24 h for 5 days. Medium supplemented with 100 μM Tegafur was then added in the liver compartment and after metabolism, the valve was opened to allow controlled diffusion of metabolites and evaluate the viability of tumor cells with a LIVE/DEAD assay. Viability tests validated the hypothesis that Tegafur was converted into the active metabolite 5-fluorouracil by MPCCs in the liver compartment, exerting the expected toxic effect on HCT-116 cells. Thus, the valve-based system allows for controlled diffusion of liver-metabolized drugs while excluding cell-cell interactions and eliminating convective transport, demonstrating the ability of such platform to study the effect of liver metabolism on anti-tumor drugs.

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Presentation: Poster

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An *in vitro* model of proteinuria for high throughput drug discovery

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Chronic kidney disease (CKD) is characterized by a progressive loss of kidney function, believed to affect ~9.1% of the global population. There is no cure for CKD, current therapies limit load on the kidney through blood pressure modulation and anti-inflammatory medications. The red flag for a clinician is the appearance of protein in the urine (proteinuria). The glomerulus is the structure in the kidney that filters waste products and small molecules from the blood into the urine, and the size-selective sieve is formed by podocytes, which have long finger-like processes that wrap around the blood vessels. Damage to these cells effectively punches holes in the filter, permitting the passage of larger and larger proteins from the blood into the urine, resulting in proteinuria. Being a 3D process, experiments to examine proteinuria typically use live rodents.

We have developed an *in vitro* 3D model of the glomerular filtration barrier (GFB), using primary podocytes derived from human kidneys. We have developed a serial culture method for podocyte isolation which yields a ~90% pure population. The cells express all known podocyte markers, including synaptopodin, WT1, nephrin, NEPH1, podocin, CD2AP, and podocalyxin. The podocytes also have central cilia, shown by pericentrin and acetylated tubulin staining. Cells are grown to TEER in Corning Transwell cell culture inserts, and we have developed an *in vitro* proteinuria assay, which we have used to demonstrate that our model retains the size selectivity of the *in vivo* GFB using a range of FITC-labelled dextran molecules of differing molecular weights and charges. We have shown that this size selectivity is disrupted using known podocyte toxins, adriamycin and puromycin. Our model is constructed in a 96-well plate format, making it amenable to high throughput analysis of GFB function and drug screening of compounds that affect GFB permeability.

Presentation: Poster

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Microfluidic high-throughput screening platform to screen pre-clinical stage compound effects on neurite outgrowth of human motor neurons post injury

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Traumatic spinal cord injury (SCI) affects millions of people of all ages around the world and can potentially lead to irreversible cognitive and motor damage without any immediate therapeutics available. There is a significant demand for physiologically relevant models of SCI. The major modeling challenge is to accurately perform the injury only on the neurites without affecting cell viability and to apply the pre-clinical stage compounds on cell soma only. Microfluidic chip designs applied to neuroscience research are based on connection between compartmentalized neuronal populations through guiding neurite outgrowth by using microchannels. The longitude of microchannels acts as a selective barrier for the exclusive passage of axons over dendrites and it promotes the unidirectional growth of the neurites from one compartment to the other.

We will present the design of a compartmentalized microfluidic device with three compartments capable of both inducing a localized axotomy and an isolation of soma and axons. Human-induced pluripotent stem cell-derived motor neurons are maintained up to 7 weeks *in vitro*. We performed a chemically induced axonal injury on isolated axons in the middle compartment only. We then follow the dynamics of the axonal regeneration using the triangular methodology with and without pharmacological compounds in soma channel. We show that this model can be successfully used for quantitative analysis of neurite outgrowth dynamics. To further strengthen the efficacy of those strategies, we will present a new technical feature with addition of a PDMS layer in which the microgrooves are hollowed out. Our data show an increase in the rate of axonal projections into triangular channel and an improvement in axon orientation and elongation.

In conclusion, this study shows the added value of neurofluidic design strategies for the investigation of neurite length dynamics and can be used for high-throughput drug-induced axonal regeneration screening for preclinical stages pharmaceutical compounds.

Presentation: Poster

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Neural network analytics as a biomarker for preclinical brain-on-chip assays

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During the past decade, the advances in organs-on-chip technology have enlarged our understanding of tissue development, organ physiology, and disease etiology; in addition, human or animal-based organs-on-chip approaches have bridged the gap between *in vitro* and *in vivo* studies by replicating at the anatomical structure and the biological function of different organs. On the macroscale, the whole-brain neuroimaging studies suggest that the anatomical alterations in the brain, particularly in neurodegenerative diseases have functional consequences. Graph-theory approaches applied on cortical parcellation derived from DTI tractography can track the early effects of pathology prior to the emergence of severe dementia on the structural level. Yet, on the microscale, the spatiotemporal reconfiguration patterns in the context of Functional Connectivity (FC) in brain-on-chip has remained largely unknown and is needed to assess functional recovery after application of a compound during preclinical trials. Here, we have developed a platform using network science metrics with the aim to analyze the FC reorganization of the human brain-on-chip-based assays with multi-compartments using Multielectrode Array (MEA). We have shown that the graph theory-based metrics can serve as a powerful tool to assess the functional neural network alterations across case-control and longitudinal studies *in vitro*.

Presentation: Poster

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All-in-one microfluidic design to integrate vascularized tumor spheroid into high-throughput platform

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The development of a scalable and highly reproducible *in vitro* tumor microenvironment (TME) platform still sheds light on new insights into cancer metastasis mechanisms and anticancer therapeutic strategies. Here, we present an all-in-one microfluidic design that integrates vascularized tumor spheroids into a highly reproducible, high-throughput platform (All-in-One IMPACT). This device allows the formation of self-assembled cell spheroids on a chip by applying the hanging drop method to the cell culture channel. Then, when the hydrogel containing endothelial cells and fibroblasts is injected, the spheroid inside the droplet can be patterned together in three dimensions along the culture channel. In just two steps above, we can build a vascularized TME within a defined area. This process does not require specialized user skill and minimizes error-inducing steps, enabling both reproducibility and high-throughput of the experiment. We have successfully demonstrated the process, from spheroid formation to tumor vascularization, using patient-derived cancer cells (PDCs) as well as various cancer cell lines. Furthermore, we performed combination therapies with Taxol (paclitaxel) and Avastin (bevacizumab), which are used in standard care for metastatic cancer. The All-in-One IMPACT is a powerful tool for establishing various anticancer treatment strategies through the development of a complex TME for use in high-throughput experiments.

Presentation: Oral

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Multi-organ experiments scaled up

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The next step towards more biomimetic *in vitro* models is the design of multi-organ devices which allow communication of different tissue types. Combining physiologically relevant organ models in perfusion systems bears technological challenges and often leads to complicated culturing setups. Complex systems require trained personnel, reduce reproducibility, and make integration into scalable routine processes difficult.

We have engineered a multi-organ platform in 384 plate-format produced completely out of polystyrene and complying with SLAS/ANSI-standard dimensions. Microfluidic channels and chambers were engineered for culturing of microtissue spheroids under physiological flow conditions. It includes 24 parallel channels, with each channel containing up to 12 spheroid compartments. The compartments have minimal dead volume (< 2 µL) and are directly accessible with robotic pipet tips for fully automated spheroid loading and retrieval. Open media reservoirs are located at both ends of each channel. Perfusion flow is generated through tilting the device back and forth on an automated system inside an incubator. Multiple devices can be operated in parallel increasing the number of conditions and statistical replicates executed in parallel.

The concept allows on-demand interconnection of up to 12 same or different spheroids per channel in a very flexible way. With the broad range of available spheroid-based organ-models, a variety of pre-clinical testing applications can be generated using the very same platform. Using the system, we were able to demonstrate that liver and islet microtissues showed significantly higher functionality under flow conditions compared to static culturing. The metabolic function of liver has been used to activate prodrugs and study their effect on tumor in liver-tumor co-cultures. Liver-islet interactions are currently investigated for metabolic disease models and investigate the influence of glucose-stimulated insulin secretion on liver metabolism.

Presentation: Oral

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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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Current aerosol exposure systems suffer from the limitation that they expose only discrete parts of the human respiratory tract in static *in vitro* cultures to a fraction of a complex aerosol. This limits the predictive power of the generated data for respiratory and systemic human effects caused by such complex aerosols.

Philip Morris International (PMI) developed the Independent Holistic Air-Liquid interface Exposure System (InHALES) as a mechanical replica of the whole human respiratory tract. It is an aerosol delivery device that perfectly matches the architecture and respiratory characteristics of the human respiratory tract, and it allows cell cultures to be exposed at the air-liquid interface in a model of each relevant respiratory tract compartment. PMI engineered InHALES to implement TissUse's proprietary microphysiological HUMIMIC multi-organ-on-a-chip (MOC) platform, which allows functional human tissue architecture homeostasis at minute scale *in vitro*. We further developed a novel HUMIMIC chip for plug-and-play insertion into InHALES for maintaining and culturing a human cell culture insert based a lung model together with other tissue constructs or organ equivalents (e.g., the liver). We have now demonstrated the integrity and viability of the lung model by CellTrace™ Calcein red orange AM and CellTox™ Green staining.

HUMIMIC-InHALES supports the development of any systemic assay with aerosol exposure, including acute and chronic toxicity and long-term disease treatment efficacy. Its combination of aerosol delivery testing for advanced cell culture systems and cutting-edge microfluidics microphysiological systems enables physical mimicry of the interconnection of cell types and systemic delivery of aerosols. The combination of lung models with liver equivalents in this novel test platform will allows us to assess the local effects of aerosols on the biological barrier of lung epithelia, entry into blood circulation, and eventual systemic effects in a single system.

Presentation: Oral

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Recapitulating glucose metabolism in a human 3D liver *in vitro* model for its application in a multi-tissue metabolic disease MPS

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There is a close link between metabolic liver diseases, type 2 diabetes, and obesity with severe changes in glucose and lipid metabolism. Understanding the mechanism of inter-tissue 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 characterized a 3D human liver *in vitro* system to recapitulate glucose metabolism and to serve as a cornerstone of a multi-tissue metabolic disease MPS.

We have cultured primary human 3D microtissue spheroid (hLiMTs) consisting of a hepatocyte-Kupffer cell co-culture by re-aggregating fully differentiated donor cells in a 96-well format under (patho-)physiological insulin and glucose conditions. After a defined disease-inducing pre-conditioning phase, insulin and glucagon sensitivity of hLiMTs was assessed by quantifying glycogen content and gluconeogenesis (GNG) upon hormonal stimulation.

Glucagon induced glycogen storage depletion in hLiMTs. Additional, assay conditions were optimized to quantify GNG by measuring glucose release from fasted hLiMTs. The amount of glucose produced through GNG increased in the presence of increasing concentrations of glucagon. In contrast, the presence of insulin resulted in reduced glucose release from hLiMTs, indicating an inhibition of GNG. Importantly, insulin resistance was observed in hLiMTs pre-conditioned in high glucose and high insulin with and without FFA.

The presented human liver model correctly replicates the hallmarks of glucose metabolism in a highly scalable format. It constitutes a one of the three central elements for building a multi-tissue MPS besides pancreatic islets and adipose microtissues. This work completes the next step towards the development of a multi-tissue model of glucose and lipid homeostasis and its application as a drug discovery tool to identify anti-steatotic and anti-diabetic drugs.

Presentation: Poster

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A microphysiological system representing liver fibrosis, the concept of AOP-chip

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Liver fibrosis is a common endpoint from chronic liver damage caused by toxicants, viral diseases, or metabolic imbalances. A hallmark of these processes is sustained hepatocellular damage that leads to a sequence of events described in the liver fibrosis adverse outcome pathway (AOP #38). Here, in the context of a transdisciplinary collaboration, we aim to develop a suitable microphysiological system (MPS) that can accurately reproduce this sequence of events and the multicellular processes leading to liver fibrosis. For this innovative application, the blueprint of the MPS mimics the graphic representation of the described AOP. Hence, the model is based on the three major cellular players in liver fibrosis: human cell lines representing hepatocytes (HepaRG), Kupffer cells (THP-1), and hepatic stellate cells (hTERT-HSC). In the presented AOP-Chip, these cell types are sequentially interconnected by microfluidics. This innovative concept should allow the quantification of individual cellular responses considered as key events (KE). This is crucial for relevant risk assessment, as not every hepatocellular damage leads to fibrosis. Indeed, preliminary data suggest that this cell-based approach allows to differentiate the effects from acute vs. chronic hepatocellular toxicants and indicate that fibrotic and non-fibrotic responses can be distinguished. The ultimate goal of the AOP-Chip is the determination of thresholds of concern or points of no return for identified key events (KE). In this presentation, we will discuss preliminary results as well as the technical and conceptual hurdles behind the design and implementation of the AOP-Chip approach.

Presentation: Oral

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Application of a throughput amenable immunocompetent microphysiologic hematotoxicity model to drug development

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With the evolution of drug modalities being explored by pharma comes the necessity to evolve the tools we use to discover them. Toward this end, we report on development and implementation of what we understand to be an elegant, cost-effective, and clinically relevant immunocompetent *in vitro* solution toward identification of, and lead optimization around, hematopoietic liabilities including anemia, thrombocytopenia, neutropenia, and lymphopenia – with additional immunogenicity read-outs – throughout the drug development landscape. By relying on primary human whole bone marrow mononuclear cells, highly quantitative 25-parameter flow cytometry, and an optimized multilineage hematopoietic differentiation cytokine cocktail, we can simultaneously investigate diverse hazards and the mechanisms driving them, across a diverse array of drug modalities. For example, using this model we evaluate hazards of nascent technologies such as antisense oligonucleotides (ASOs), T-cell bispecifics (TCBs), as well as more classic small molecule therapies such as BCL-class anti-apoptotic inhibitors and report on highlighted endpoints. Additional use-cases of this model are currently under exploration and include iron-metabolism investigations, single model safety:efficacy therapeutic index calculations, as well as structure-activity relationship-based lead optimization for non-life threatening indications. Future directions will focus on personalized healthcare by integrating *in vitro* read-outs with a quantitative systems pharmacology model, using, for example, patient-derived myeloma material for identification of optimal drug dosing regimens and response prediction with a strong emphasis on quality-of-life metrics.

Presentation: Poster

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uScar: A mechanically active model of human cardiac fibrosis on chip

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Myocardial infarction represents globally one of the leading causes of death. After the cardiac insult, the initial physiological wound healing process often evolves into cardiac fibrosis. The pathology is characterized by a phenotypical fibroblast transition into myofibroblasts and by abundant extracellular matrix (ECM) deposition, which thickens and stiffens heart walls impairing its functionality. Since no treatment is currently available for cardiac fibrosis, the development of *in vitro* scar-models is crucial for identifying possible therapeutic targets. We present a scar-on-chip model (uScar) able to mimic the key steps of scar formation (fibroblast proliferation and activation, ECM remodeling) by the sole application of mechanical stimulation provided to 3D cardiac fibroblast microtissues (reducing confounding effects of exogenous morphogens).

Human atrial cardiac fibroblasts (hA-CFs) were embedded in fibrin hydrogel and cultured with uBeat® platfom for 7 days (Visone et al., 2021). Mechanical (i.e., cyclic 10% uniaxial strain, 1Hz), chemical (i.e., 5 ng/mL TGFβ1) or combined stimulations were provided to microtissues to assess the onset and progression of the fibrotic process. The cyclic mechanical stimulation, alone or combined with TGF-β1, significantly enhanced fibroblast-to-myofibroblast transition (increased α-SMA expression). Upregulation of COL1A1 and COL3A1 gene expression was highlighted in both chemically and mechanically stimulated microtissues. An increased production of Collagen I and Fibronectin was detected in all tested conditions, but static microtissues. Aggrecan deposition was instead specifically triggered by mechanical stimulation.

Drug screening studies on uScar highlighted the positive effects of tested compounds (e.g., Tranilast, Pirfenidone) in preventing myofibroblast transition and ECM deposition. uScar was also exploited to test advanced therapeutics (Paoletti et al., 2020) (microRNAs for direct reprogramming of fibroblasts into cardiomyocytes by novel lipoplexes) in a native-like mechanically active environment.

In summary, uScar model recapitulates the different steps of fibrotic process *in vitro* and was validated as promising tool to test both pharmacological and advanced therapies.

Presentation: Oral

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HMGB1 enriching amnion epithelial cell-derived exosomes induce preterm birth phenotype within the feto-maternal interface organ-on-chip (FMi-OOC)

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Preterm birth (PTB; < 37 weeks of gestation) impacts ~11% of all pregnancies and contributes to 1 million neonatal deaths worldwide annually. An understanding of the feto-maternal (F-M) signals that initiate birth at term is critical to design strategies to prevent their premature activation resulting in PTB. Although endocrine and immune signaling are well-reported, fetal-derived paracrine signals capable of transitioning quiescent uterus to an active state are poorly studied. Recent reports have suggested that senescence of the fetal amnion membrane coinciding with fetal maturation generates inflammatory signals capable of triggering parturition. This is by increasing the inflammatory load at the feto-maternal interface (FMi) tissues (amniochorion-decidua). High mobility group box 1 protein (HMGB1) is one of the inflammatory signals released by senescent amnion cells via extracellular vesicles (exosomes; 40-160 nm). This study tested the hypothesis that senescent amnion cells release HMGB1, predisposing the FMi to parturition. To test this hypothesis, exosomes from amnion epithelial cells (AECs) grown under normal conditions were engineered to contain HMGB1 by electroporation (eHMGB1). eHMGB1 was characterized, and its propagation through the FMi was tested using a four-chamber microfluidic organ-on-a-chip device (FMi-OOC) that contained four distinct cell types (amnion and chorion mesenchymal, chorion trophoblast, and decidual cells) connected through arrays of microchannels. In the OOC model, eHMGB1 propagated through the fetal cells and matrix to the maternal decidua and increased inflammatory receptors and cytokines. Furthermore, intra-amniotic injection of eHMGB1 (containing 10 ng) into pregnant CD-1 mice on embryonic day 17 led to PTB, as predicted by the FMi-OOC model results. In conclusion, this study determined that fetal exosome-mediated paracrine signaling can generate inflammation and induce parturition. Importantly, *in vivo* functional validation of the FMi-OOC experiments using an animal model demonstrated the predictive capability of OOC models in testing physiologic and pathologic conditions as seen *in vivo*.

Presentation: Poster

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An interconnected fetal membrane and placenta organ-on-chip model

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Drug testing in obstetrics is challenging as two patients, the mother and the fetus, must be evaluated. Current *in vitro* and commonly used *in vivo* animal models structurally or functionally do not mimic the human feto-maternal interface (FMi), contributing to this challenge. To overcome this limitation, we developed the first multi-organ organ-on-chip (OOC) model that represents the structure, functions, and responses of both human FMis (fetal membrane and placenta). The fetal membrane part of the OOC (FMi-OOC) contains four concentric circular cell chambers (decidua, chorion trophoblasts, and amnion [mesenchyme and epithelium]) cells, also incorporating collagen matrix. The placenta part of the OOC (PLA-OOC) is comprised of three rectangular chambers containing syncytiotrophoblasts, cytotrophoblasts, and human umbilical vein endothelial cells (HUVECs). Here, both trophoblast layers formed natural 3D cell barriers to mimic the placental barrier function. Each cell culture chamber of the FMi-OOC and PLA-OOC is interconnected by an array of microchannels that provide control over cell isolation and fluidic diffusion while allowing cell migration that can be visualized microscopically. Cells cultured in the OOC models were characterized by cell-specific markers (immunocytochemistry) and morphology analysis (microscopy), while media diffusion rates were established using fluorescent dye diffusion analysis. A therapeutic dose of rosuvastatin (200 ng/mL) was used to validate the utility of the interconnected OOC model for preclinical trials (LC-MS/MS). OOCs mimicking both FMis maintained cell morphology, specific markers, barrier functions, nascent collagen production, and viability over 72 h period. While media diffusion occurred over 72 hours (dye propagation), rosuvastatin traversed through cells within 4 hours, documenting OOC's ability to test the pharmacokinetics of drugs. These results show that the OOC platform provides novel tools to study physiological and pathological pregnancy conditions and can be used to conduct preclinical trials and toxicology screening, minimizing the need for animal testing.

Presentation: Poster

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Modeling ascending infection and inflammation through the maternal uterine tract using a vaginal-cervix-decidual organ-on-a-chip

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A vagina-cervix-decidua-on-a-chip (VCD-OOC) mimicking the maternal uterine tract was developed to study ascending bacterial infection and its impact on the decidua that can lead to preterm birth. We developed the VCD-OOC composed of six cell culture chambers interconnected by arrays of microchannels to mimic the lower female genital-maternal uterine tract. The six chambers represent the vagina, ectocervix, transformation zone, cervical stroma, endocervix, and decidua, and were populated by vaginal epithelial cells, cervical epithelial cells, cervical stromal cells, and decidual cells. Cells cultured within the VCD-OOC were characterized by morphology and immunostaining for intermediate filaments and cell-specific markers. An ascending *Ureaplasma parvum* (*U. parvum*) infection was created in VCD-OOC by inoculating *U. parvum* (low dose: 107 color-changing units [CCU]; high dose: 1010 CCU) in the vagina chamber. *U. parvum* propagation was monitored for 48 hours post-infection (live microscopy), with their cytotoxicity (Lactate Dehydrogenase [LDH] assay) and inflammatory effects (multiplex inflammatory cytokine assay) in the maternal and fetal cells analyzed. Cellular characteristics (morphology, collagen production, expression of cell-specific markers) in the VCD-OOC mimicked those seen in the lower female genital-maternal uterine tract tissues, validating the physiological relevance of our OOC model. *U. parvum* reached the cervical epithelial cells and decidua within 48 hours and did not cause cell death in any of the VCD-OOC cells. Low-dose *U. parvum* vaginal infection increased pro-inflammatory cytokines in cervical stromal cells but did not promote inflammation in decidua cells. However, high-dose *U. parvum* infection increased pro-inflammatory cytokines in ectocervical epithelial, cervical stromal, and decidual. Using our OOC models of the lower genital tract-decidual interface, we established an *in vitro* model of ascending *U. parvum* infection. We report colonization of *U. parvum* in various cell types; however, inconsistent and low-grade inflammation seen was suggestive of poor immunogenicity induced by *U. parvum*, similar to clinical findings.

Presentation: Poster

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Modeling preterm birth *in vitro* using a feto-maternal interface organ-on-chip

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Infection and inflammation during pregnancy contribute to preterm birth (PTB). Two inflammatory pathways can lead to PTB: ascending maternal infection (maternal-to-fetal), and intraamniotic infection followed by a fetal inflammatory response (fetal-to-maternal). However, propagation of infectious and inflammatory mediators across the feto-(fetal membrane)-maternal (decidua)-interface (FMi) is difficult to study due to the lack of appropriate models. To study infectious agent's propagation kinetics, decreased Human Leukocyte Antigen (HLA)-G expression, and promotion of Epithelial-to-Mesenchymal Transition (EMT), we developed a microfluidic FMi-organ-on-chip (FMi-OOC) device that can mimic the structure and functions of the FMi. The FMi-OOC is designed to have four-circular cell culture chambers, containing one cell type from the maternal side and three cell types from the fetal side, interconnected by Type IV collagen-filled microchannels, mimicking the structure of FMi *in vivo*. Lipopolysaccharide (LPS, 100 ng/mL), a proxy for infectious microbial pathogens, was added to either the decidua or amnion epithelium chamber to model either maternal ascending infection or intra-amnionic infection. Cellular changes (immunocytochemistry-LPS, Toll-like receptor 4 (TLR-4), EMT (vimentin/cytokeratin-18), HLA-G) and inflammatory changes (multiplex cytokine immunoassay) were monitored. In both infection models (maternal-to-fetal and fetal-to-maternal), LPS propagated from one cell type to the other within 72 hours. LPS propagation in both models induced TLR-4 expression in each cell type in a time-dependent manner. Maternal LPS exposure, but not fetal, significantly decreased HLA-G expression in the chorion. Compared to untreated controls, both decidual and amnion exposures of LPS induced time-dependent and cell-type-specific IL-6 and GM-CSF production in FMi cells. Conversely, fetal infection and generation of IL-8 was associated with EMT. This OOC model provides a novel platform to study physiological and pathological processes at the FMi and is expected to have broad utility in the field of obstetrics.

Presentation: Oral

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Induced pluripotent stem cell-derived neural organoids incorporating microglia for interrogation of neural toxicity

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There is a critical need to develop advanced neural microphysiological systems (MPS) that can complement and reduce the use of animal models and more accurately predict efficacy and toxicology in drug discovery. Most current *in vitro* models, however, do not reflect the complexity and diversity of cell populations and cell-cell interactions found within neural tissue. To address these limitations, Stem Pharm has leveraged its proprietary synthetic hydrogel platform to enable the formation of reproducible induced pluripotent stem cell (iPSC)-derived neural organoids containing microglia and vascular cells. The neural organoids are formed in 96-well plates from iPSC-derived neural precursor cells, microglia, endothelial cells, and mesenchymal stem cells and are ready for screening 21 days after initial plating. Single cell transcriptional analysis demonstrates that the organoids are cell-type diverse, containing multiple neuronal subtypes, astrocytes, microglia, and endothelial cells. Incorporated microglia are distributed throughout the organoids, display ramified morphology resembling morphology of *in vivo* microglia, and demonstrate a gene expression signature that strongly correlates with *in vivo* microglia expression. To demonstrate application for toxicology screening, organoids were subjected to a screen of known neurotoxins, including chlorpyrifos, an organophosphate insecticide; lead acetate, which is known to cause developmental defects; Valproate (valproic acid, VPA), an anti-convulsant; and PLX-3397, a CSF1R inhibitor which has been shown to be toxic to microglia. Transcriptional profiles between control and treatment groups were analyzed and differential expression (DE) analysis between treatment groups was performed to identify gene ontology (GO) sets affected by compound treatment and to compare with publicly available data sets. Our results demonstrate the importance of using multicellular systems to assess toxicities, the promising application of Stem Pharm's neural organoids for toxicology screening, and the broader potential for facilitating translation between pre-clinical and clinical discovery and development.

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Induced pluripotent stem cell-derived neural organoids incorporating microglia for interrogation of neural inflammation

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Neuroinflammation is a complex response to brain injury involving activation of the innate immune response, release of inflammatory mediators, and the generation of reactive species resulting in downstream effects including vascular compromise, oxidative stress, and neurotoxicity. Neuroinflammation is a critical component in the etiology and progression of many diseases including neurodegenerative diseases, stroke, trauma, seizures, neuropsychiatric disorders, and brain cancers. There is a critical need to develop advanced neural microphysiological systems (MPS) that can model neuroinflammation and bridge the gap between simplistic cell models and clinical data. Stem Pharm has leveraged its proprietary synthetic hydrogel platform to enable the formation of complex, reproducible, induced pluripotent stem cell (iPSC)-derived neural organoids containing microglia and vascular cells that are well-suited to study neural inflammation. The neural organoids are formed in 96-well plates from iPSC-derived neural precursor cells, microglia, endothelial cells, and mesenchymal stem cells and are ready for screening 21 days after initial plating. Single cell transcriptional analysis demonstrates that the organoids are cell-type diverse, containing multiple neuronal subtypes, astrocytes, microglia, and endothelial cells. Bulk and single cell RNA-seq analysis demonstrates high intraclass correlation and low coefficients of variation between biological replicates. Incorporated microglia are distributed throughout the organoids, display ramified morphology resembling *in vivo* morphology, and demonstrate a gene expression signature that strongly correlates with *in vivo* microglia expression. Modulation of microglia within the organoids to pro- and anti-inflammatory phenotypes was validated through stimulation with lipopolysaccharides, interferon gamma, TGFβ & IL-10, or IL-4 & IL-13 and incorporation of patient-derived glioblastoma cells. These data demonstrate the promising application of Stem Pharm's advanced neural organoids for facilitating translation between pre-clinical and clinical discovery and development in the active area of neuroinflammation.

Presentation: Oral

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Advanced synthetic biomaterials that enable microphysiological systems

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Modeling cellular and tissue microenvironments is a key component of developing more physiologically relevant microphysiological systems. Stem Pharm has developed a synthetic hydrogel platform that permits optimization of substrates for cell expansion, differentiation, and screening applications. We provide application-specific biomaterials for advanced cellular assay platforms through control of the substrate mechanical properties, adhesion ligand presentation, and utilization of chemistries that maintain cellular health and function. The Stem Pharm hydrogels incorporate multi-armed polyethylene glycol (PEG) functionalized with norbornene to enable thiolene-mediated incorporation of bioactive peptides. Design of Experiments methodology is employed to identify hydrogel formulations that support a variety of applications including assessment of vascularization potential, culture of tumor organoids, neurite outgrowth, co-culture models and the formation of neural organoids. Our vascular tubulogenesis hydrogel enables high throughput screening (HTS) for vascular disruptors using human umbilical vein endothelial cells or iPSC-derived endothelial cells. Use of this hydrogel provides advantages over current assay platforms that use Matrigel. Matrigel is a complex mixture of proteins that is challenging in an HTS workflow due to its temperature sensitivity and lot-to-lot variability. When compared to Matrigel, the hydrogel-based vascular tubulogenesis assay demonstrates improved Z' and is amenable to a 384-well plate screening format. Our neural differentiation and maturation substrates couple enhanced maturation and neurite outgrowth with ease of use compared to traditional neural substrates. The Stem Pharm platform is flexible and suitable for co-culture and organoid applications, allowing cells to be embedded within or plated on top of the hydrogel to best meet the requirements and workflow of the desired application.

Presentation: Poster

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Ex vivo human 3D NASH model as a screening-based discovery approach for selecting and prioritizing drug candidates

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Non-alcoholic fatty liver disease (NAFLD) is an emerging chronic liver disease characterized by hepatic steatosis that often progresses into steatohepatitis (NASH). Poor translation of animal studies to humans have resulted in a lack of approved NAFLD/NASH-specific drug therapies. We have modeled NAFLD/NASH *ex vivo* using human microtissues technology as a high-throughput tool for drug discovery.

We generated liver microtissues by culturing human primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells in InSphero plates. Upon exposure to defined lipotoxic and inflammatory stimuli, including free fatty acids and LPS in media containing high levels of sugar and insulin, this 3D NASH model displayed pathophysiologically relevant features within 10 days of treatment. Methods for assessing characteristic markers for NASH included accumulation of intracellular triglycerides (bioluminescent assay), secretion of pro-inflammatory cytokines/chemokines (Luminex), and secretion of pro-collagens type I/III (HTRF/ELISA). Quantification of fibrosis based on SiriusRed-stained tissue slices was performed using PharmaNest AI.

We observed increases in intracellular triglyceride content and secretion of proinflammatory (e. g. IL-6, IL-1b)/profibrotic (e.g., IL-10, MCP-1) cytokines/chemokines in NASH-treated tissues compared to the untreated controls. We detected increased collagen deposition, and increased secretion of procollagen type I/III peptides under NASH conditions. Whole transcriptome analysis of NASH-treated tissues versus control revealed differential regulation of genes associated with lipid metabolism, inflammation, and fibrosis induction. Treatment with anti-TGF-β antibody and ALK5i (TGF-βRI inhibitor) decreased pro-collagen type I/III secretion. Decreased collagen deposition based on quantification of fibrosis of Sirius Red-stained tissues was observed in the presence of anti-TGF-β antibody and ALK5i. Results from biochemical readouts of the NASH-treated tissues with drug clinical candidates (Selonsertib/Firsocostat) were in line with clinical observations.
This high-throughput 3D human NASH model represents a promising approach for NASH drug efficacy selection early within the drug discovery process.

Presentation: Oral

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Efforts to assess the technical requirements based on unmet needs that MPS should have for social implementation

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MPS, which mimics the cellular environment of tissues and organs, is expected to be a culture technology that solves the unmet needs for *in vitro* evaluation in new drug development. At the World Summit of MPS and other similar symposia held so far, there has been a lot of discussion on what should be considered for social implementation of MPS. However, no common view has been presented on the specific issues regarding the technical requirements to be solved for social implementation. In this presentation, based on the discussions in the AMED-MPS project (Ishida, 2021) in which the presenter has participated, the concept of technical requirements for social implementation of MPS based on unmet needs will be presented from the perspective of regulatory science. From user unmet needs, evaluation points are derived. The technical requirements are the elements that the MPS should have in order to realize the assays specified by the evaluation points. The culture methods used to accomplish the endpoints can be complex, but when they are broken down to the technical requirements that comprise the assay, these technical requirements are consisted of simple elements. We would like to discuss the eligibility as technical requirements by taking up specific evaluation cases of culture media, cell adhesion to culture vessels, and adsorption of test substances to culture substrates, which are being discussed in the project.

Reference

Ishida, S. (2021). Research and development of microphysiological systems in Japan supported by the AMED-MPS project. *Front Toxicol 3*. doi:10.3389/ftox.2021.657765

Presentation: Poster

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Efforts of the physiological culture environment reconstitution *in vitro* necessary for the development of MPS

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The MPS has been developed as "a culture device that can mimic the blood flow *in vitro* and elucidate its mechanical effects on cells as well as its effects of mass transfer of nutrients, metabolites, or drugs." However, due to the fact that its development originated in Micro Electro Mechanical Systems (MEMS) technology, the substrate of the cell culture surface has not yet been sufficiently studied. In some cases, the bottom of the culture chamber is flat, just like a normal culture plate. In this presentation, I would like to introduce the effects of cell culture substrates on cell functions that have been developed in Japan. We would also like to discuss the role of the substrate on the culture surface, using our own research as an example. When hepatocytes are cultured in three-dimension using Cellbed™ (Japan Vilene), the formation of bile canaliculi is promoted and transporters involved in bile excretion are localized on the bile canaliculi. In addition, hepatic stellate cells are activated when cultured on a culture plate and become close to the pathological state, but when cultured in three-dimension using VECELL (Cosmo Bio), they are deactivated and become close to the normal cellular state (Horiuchi et al., 2018). Both culture methods bring out cell functions that have been difficult to achieve in two-dimensional culture, and it is expected that similar culture methods will be incorporated into MPS.

Reference

Horiuchi, S., Kuroda, Y., Fujii, R. et al. (2018). Deactivation of hepatic stellate cells by culturing on VECELL inserts. *AATEX 23*, 53-62.

Presentation: Poster

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A novel multi-organ *in vitro* model for combined and more predictive toxo-efficacy assays

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Introduction: Many human disorders display unknown tissues relationships affecting disease progression and drugs response. While *in vitro* assays fail in recapitulating organ-organ connection, animal models reveal species-specific drug outcomes. To this purpose, a novel multi-organ microphysiological system (MIVO®) has been adopted to fluidically connect 3D ovarian cancer tissues with a hepatic cellular model and simulate the systemic cisplatin administration for investigating its anticancer efficacy and simultaneously measuring potential hepatotoxic effects.

Methods: Human hepatocellular (Hep-G2) and ovarian cancer (SKOV-3) cells line have been used to realize liver models and 2D/3D tumor model. Computational fluid-dynamic modeling has been performed to simulate the capillary blood velocity that was set-up within MIVO®, where 3D ovarian cancer and the liver model were cultured fluidically connected. First, a drug concentration (10-100 μM) screening was performed by using 2D and 3D single organ models. Then, drug efficacy and toxicity assays were performed in MIVO® and compared with both static co-cultures and single organ models. Ovarian and liver cells death, half maximal effective concentration (EC50) and median lethal dose (LD50) for Skov-3 cells and Hep-G2 cells were quantitatively assessed. Cisplatin effects were also qualitatively assessed by immunofluorescence.

Results: A linear decay of Hep-G2 and Skov-3 cells viability was observed with increasing cisplatin concentration after 48 h of treatment. Furthermore, the 3D ovarian cancer model demonstrated higher drug resistance than the 2D model. Finally, reduced efficacy against the 3D ovarian cancer tissue and hepatotoxicity were observed in the MIVO® compared with single organ model (0.5X of EC50 and 2X of LD50).

Discussion and conclusions: Results highlight that the introduction of 3D cells culture and multi-organ fluidic connections resembling the *in vivo* conditions significantly impact in both drug efficacy and toxicity outcomes, indicating the importance of developing more predictive pre-clinical tools for drug screening.

Presentation: Poster

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Anaerobic microbiota-derived extracellular vesicle research by PDMS-free gut on a chip

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The gut microbiota and their products have a critical role in human health and are involved in all physiological processes. One of the ways in which these processes in the body are potentially influenced by the microflora is through extracellular vesicles (EVs) and the RNA content in them, but currently, research methods for these processes are limited. One of the most promising new methods currently in microflora research is the gut-on-chip (GoC) platform. Therefore, the aim of our research is to study EV RNA content of cancer patient microbiome, which can enter from lumen to circulation by applying GoC devices. To that end, we have currently developed a PDMS-free GoC device since PDMS have high gas permeability, which is unsuitable to recapitulate the O_2 gradient between gut lumen and endothelial channel. Next, we successfully optimized anaerobic microbiota isolation and co-cultivation within stable cell lines in PDMS-free GoC from human stool samples, which were confirmed by metagenome sequencing data. Currently, we are analyzing EV RNA content of gastric cancer patient-derived microbiota and how this content differs between EV retained in the gut lumen and those that can pass through the gut-endothelial barrier.

Presentation: Poster

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Functional enhancements and crosstalk of liver-small intestine in a micro-stirrer-based on-chip perfusion MPS with direct oxygenation

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Conventional *in vitro* models have limited physiological relevance, due to the lack of functional cell sources and their interactions under physiological environments. However, intra- or inter-organ interactions are important factor affecting various phenomena such as metabolic activity and systemic response in human body. Especially in the liver-intestine axis, although the effect of such organ interactions (crosstalk) has been demonstrated *in vitro*, the mechanism is still unclear. Understanding this mechanism would give valuable insight in drug development or prediction of human body systemic response.

To elucidate liver-intestine crosstalk, *in vitro* culture system using MPS would be ideal. In this study, we cocultured human iPSC-derived intestinal epithelial cells and chimeric mouse-derived fresh human hepatocytes (PXB cells) in an open plate-type MPS with a unique on-chip pumping mechanism using a micro stirrer. We evaluated the functions and interactions of both cells. The same evaluation was performed using the MPS with oxygen permeable membrane at the bottom of the culture wells.

On the gut side, increase in TEER and permeability were observed in the oxygen permeation and co-culture condition. In addition, gene expressions of the intestinal cells cocultured with increased oxygenation are comparable to those in the adult intestine. On the liver side, increase in metabolic activity (CYP enzymes) and gene expression are observed clearly from the increased oxygen and co-culture condition. Omics analysis using RNA-seq and metabolomics will be performed to further elucidate the crosstalk mechanism. This implies the powerful capability of the culture system using novel on-chip kinetic pump MPS as tools to study liver-intestine *in vitro* crosstalk.

Presentation: Poster

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Multifluorescent human brain organoid model for high throughput chemical toxicity and drug efficacy screening

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Developmental Neurotoxicity (DNT) is one of the most complex endpoints to address. Current *in vivo* approaches are prohibitively expensive, time consuming and have low predictivity for humans. The main goal of this project is to develop an integrated testing strategy for DNT. It is based on a human 3D iPSC-derived brain organoid model with knocked-in fluorescent tags for neural markers, where six key events of neurodevelopment and their perturbation can be assessed fast with high-content imaging in one assay. This is significant improvement of existing 3D brain organoid models. Our model will eliminate the need of a laborious antibody staining-dependent quantification and will allow to use the brain organoids for chemical and drug screening.

Here, we take advantage of CRISPR technology to insert fluorescent tags in the iPSC to tag the neuronal (beta-III-tubulin), astrocyte (GFAP), oligodendrocyte (PLP1) and synaptic specific (synaptophysin) genes upon differentiation into brain organoids (miniBrainbow). We considerably improve overall knock-in efficiency compared to previous reports. The selected clones were differentiated to neural progenitors and brain organoids for validation of the tag expression and specificity was evaluated by gene expression, flow cytometry and confocal imaging. In addition, we are working to establish and optimize the high-content imaging and image analysis pipeline that we will use to quantify the effects toxicants have in the miniBrainbows. Overall, this model provides: a) a more effective method to generate iPS reporter cell lines; b) a robust human-relevant and standardized brain organoid *in vitro* assay, which can be used in numerous applications including DNT and drug efficacy screenings; and c) standardization of the model for high-throughput phenotypic imaging will reduce the costs and accelerate chemical prioritization and testing.

Presentation: Oral

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A human lymph node-on-a-chip with functional NK cells

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Vaccine development is crippled by the lack of reliable human models to evaluate vaccine-induced immune responses in a physiologically-relevant preclinical setting. Animal models fall short as they only partially recapitulate diverse human immune interaction pathways. We initially developed a human lymphoid follicle-on-a-chip microfluidic culture device (LF Chip) that contains autologous B cells, T cells and dendritic cells (DCs) cultured in an extracellular matrix gel within one microfluidic channel while being superfused through a parallel channel, which self-assemble into functional germinal centers containing plasma cells and replicate complex human immune responses. The LF chips demonstrated improved antibody responses against split virion influenza vaccination as compared to 2D cultures, which were enhanced by the addition of a squalene-in-water emulsion adjuvant, evident with the increase in LF size and number. In addition, we have integrated human NK cells into this system to create a Lymph Node Chip (LN Chip) and have begun to use it to investigate immune responses following pharmacological interventions. The capacity of NK cells in inducing direct cytotoxic killing of infected or cancerous cells is well-established; however, their significance in cytokine production and their crosstalk with other immune cells require further exploration. Current evidence strongly suggests that an immune network, including DCs and NK cells, interacts within the inflamed lymphoid tissue local environment and determines adaptive immune response quality. Thus, LN chips containing NK cells offer an additional layer of cellular complexity and better representation of living LFs, thereby opening doors to understanding the role of NK cells in modulating adaptive immune response and inching towards a more reliable vaccine evaluation.

Presentation: Poster

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Human host-microbiome interactions and mucus physiology modeled in cervix and vagina chips

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Local interactions between commensal microbiome, host mucosal epithelium, and microenvironmental factors in the human cervix and vagina play a central role in regulation of vaginal health, and when dysregulated can lead to bacterial vaginosis, miscarriage, and preterm birth. Current understanding of these interactions rely on metagenomic analysis, microbial cultures, and *in vitro* models, which unfortunately fail to mimic the physiological mucosal microenvironment of the human reproductive tract. Here we describe Organ Chip microfluidic culture models of human cervix and vagina containing primary, hormone-sensitive, cervical or vaginal epithelium interfaced with stroma. Integration of TEER, O_2, and pH sensors in the chips allows for dynamic monitoring of changes in the epithelium barrier function, oxygen tension, and pH levels. Using this approach, we found that the Vagina Chip generates a low oxygen concentration in the epithelial channel enabling assessment of host innate immune responses to healthy versus dysbiotic bacteria. The Cervix Chip produces cervical mucus with *in vivo*-like biophysical and chemical properties and responds to changes in hormonal stimuli. Co-culture of *Lactobacillus*-based consortia in the Vagina Chip was accompanied by strain-level stability and synergy within the consortia as determined by metagenomics analysis, as well as maintenance of epithelial cell viability, increased D-lactate, pH reduction, and down-regulation of proinflammatory cytokines, all of which are observed in healthy vagina. In contrast, culturing *Gardnerella vaginalis*-containing dysbiotic consortia in the Vagina Chip resulted in epithelial cell injury, a rise in pH, and upregulation of proinflammatory cytokines. These studies demonstrate that Organ Chip technology can be used to create preclinical models of human vagina and cervix to evaluate the effects of both healthy and dysbiotic vaginal microbial consortia. Our results also suggest that multi-strain *Lactobacillus*-based live consortia may potentially be useful as probiotic therapeutics to improve vaginal health.

This work was supported by the Bill & Melinda Gates Foundation.

Presentation: Oral

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Applying a microphysiological 3D human liver-islet microtissue platform to study drug-drug interaction

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The conventional cell culture models lack the complex multi-organ interactions and dynamic drug processing in the human body that is crucial to accurate disease modeling and predictive drug testing *in vitro*. We developed a microphysiological 3D human liver-islet microtissue platform that enables direct organ cross-talk. As a proof-of-concept study, we recapitulated a well-known drug-drug interaction (DDI) phenomenon; the elevated risk of life-threatening hyperglycemia in type 2 diabetic-patients that are co-administered with antidiabetic drug gliclazide and antibiotic rifampicin. This adverse DDI is caused by rifampicin-enhanced activity of drug metabolizing CYP450 enzymes which results in increased gliclazide clearance rate. Lower gliclazide plasma levels lead to decreased plasma insulin concentrations and thus increased blood glucose levels in the patients. 3D human islet microtissues (hIsMTs) and 3D human liver microtissues (hLiMTs) were either cultured alone or together in a microfluidic multi-tissue assay plate. The plate 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 MTs. The DDI was characterized by determine gliclazide clearance in hLiMT cultures and comparing insulin secretion in liver-islet co-cultures in the absence or presence of rifampicin. Rifampicin co-treatment led to an increase in gliclazide clearance by the hLiMTs (percentage of remaining compound: 34.7 % and 53.0 % respectively 96 h ± rifampicin). Gliclazide significantly increased insulin secretion from hIsMTs. In the presence of rifampicin, insulin secretion from hIsMTs was markedly reduced, and was comparable to the level of insulin secretion without gliclazide. We have paved the way for further application of the liver-islet microphysiological platform in the fields of metabolic disease progression, drug efficacy and toxicity testing, DDI and drug clearance assays.

Presentation: Poster

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Steatosis as risk factor for drug-induced liver injury (DILI)

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The sensitizing effect of steatosis and NASH as general risk factors for human DILI is receiving a lot of attention. Reasons are the high incidence of patients with metabolic diseases in our population and the need for a more differentiated patient specific risk assessment of new drug candidates. Patients with steatosis might be more sensitive and more fragile as healthy subjects. Until now the relationship between DILI and underlying steatotic diseases has not been systematically investigated. The steatosis DILI risk factor hypothesis appears very plausible but still needs clinical endorsement, as well supportive data from *in vitro* experiments. Human 3D liver microtissue, containing scaffold free co-culture of primary hepatocytes, Kupffer cells and liver endothelial cells when exposed to free fatty acids in media, high levels of sugars and insulin displayed substantial accumulation of lipids within 7 days of treatment as detected by Nile red staining and triglyceride levels. 3D human liver microtissues have been validated by 108 clinically tested FDA-annotated DILI drugs by the determination of cellular ATP levels showing high specificity and sensitivity. It was the purpose of the present study to evaluate the cholestatic drug Chlorpromazine and the non-cholestatic drug Acetaminophen under steatosis and non-steatosis conditions in human 3D liver microtissues. Under steatosis conditions the cytotoxicity of the cholestatic drug Chlorpromazine was significantly enhanced compared to non-steatosis conditions. The cytotoxicity of the non-cholestatic Acetaminophen under same conditions was not enhanced. These preliminary results suggest that drug-induced cholestasis together with underlying steatosis can enhance DILI and might be a risk factor for the expression of DILI in patients. The increased cytotoxicity of cholestatic drugs might come from the intracellular accumulation of cytotoxic bile acids. To better understand this relationship, further investigations of cholestatic and non-cholestatic drugs under steatotic and non-steatotic conditions are necessary.

Presentation: Poster

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Development of cardiac microphysiological system for detecting anti-cancer drug-induced contractile dysfunction

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The directly mortality rate of cancer has been decreased in cancer patients, while the incidence of heart failure, a serious side effect of anti-cancer drugs, has been increased. Since many of anti-cancer drugs-induced side effects are systemic, it is not easy to detect heart-specific side effects in individual animals in the non-clinical periods. In addition, heart failure may be caused by prolonged exposure of anti-cancer drugs, and/or there may be species differences in the occurrence of side effects. It is difficult to accurately predict the heart failure risk of anti-cancer drugs in the current evaluation systems such as animal echocardiography and contractile assessment using animal hearts. Therefore, development of new *in vitro* assessment using human cells, such as human iPS cardiomyocytes, which are well understood for predicting the risk of drug-induced proarrhythmia is strongly expected.

In recent years, the development of cell culture platforms called microphysiological system (MPS) has been became active, thus we have been working on the development of cardiac MPS which can detect anti-cancer drug-induced contractile dysfunction. In this study, we constructed a contraction assessment system using three-dimensional myocardial tissue generated from human iPS cardiomyocytes (h3D heart tissue) on a polydimethylsiloxane (PDMS) micro device. We confirmed that isoproterenol, a β-adrenoceptor agonist, showed positive inotropic and chronotropic action.

Then, we examined the effects of 72-hour exposure to doxorubicin, an anthracycline anti-cancer drug, on contraction in h3D heart tissue. As a result, we observed a concentration- and exposure time-dependent impairment of contraction. Furthermore, we investigated the difference in contractile dysfunction depending on the method of exposure to doxorubicin. In the future, it is necessary to compare and verify the validity of this evaluation system with human clinical data or animal experimental data.

Presentation: Poster

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A lung-on-chip model of alveolar inflammation illustrating anti-inflammatory drug response

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During inhaled drug development and pulmonary disease modelling, pre-clinical testing is still heavily relying on animal data due to the lack of predictive and validated *in vitro* models to estimate human drug response. Complex lung-on-chip systems emulate the three-dimensional structure of the air-blood-barrier in addition to breathing dynamics and offer the possibility to model pathological conditions and predict drug response more accurately than simple *in-vitro* models.

In the presented study, we describe the development of a human drug response model of the human inflamed alveolus. Our model was set up with an immuno-competent co-culture of alveolar epithelial cells with macrophages on the AXLung-on-chip System (AlveoliX AG).

To achieve that, we co-cultured the alveolar epithelial cell line hAELVi, which has been shown to form a tight barrier comparable to the human air-blood barrier (Kuehn et al., 2016) with monocyte-derived macrophages (THP-1 cell line) as an alveolar macrophage surrogate (Kletting et al., 2018). The efficacy of anti-inflammatory treatment with glucocorticoids after inflammation was measured via apical cytokine release (bead-based flow cytometry) and barrier integrity (TEER) after 24-48 h in static and dynamic (breathing-like) conditions.

Two different approaches were compared: an inflammation triggered by bacterial LPS and one triggered by a combination of TNFα and IFNγ. In both cases, the presence of macrophages as an immune component in the model was crucial to emulate proper inflammatory responses. The treatment with glucocorticoids reduced inflammation in both models. Breathing dynamics greatly influenced the formation and stability of the alveolar epithelial cell barrier in our model. Whereas it did not influence LPS-driven inflammation, it seemed relevant for the TNFα/IFNγ-driven inflammation set-up.

Altogether, our *in vitro* model replicates key features of alveolar inflammation and shows anti-inflammatory drug response, highlighting its potential as a tool for drug development and disease modelling, while composing an alternative to animal-based studies.

Presentation: Poster

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Human tendon-on-chip (*h*ToC) platform for modeling inflammation, fibrosis, and cell cycle regulation in fibrovascular tendon healing

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Evidence indicates 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. This is consistent with the hemorrhage due to vascular injury when the tendon is ruptured, yet the crosstalk between the tendon fibroblasts (TC), endothelial cells (ECs) and inflammatory cells has thus far been understudied. We designed a modular *human* tendon-on-chip (*h*ToC) to investigate direct and paracrine signaling between ECs, TCs and circulating immune cells which are present in the healing tendon's fibrovascular scar. The *h*ToC comprises a TC laden collagen hydrogel separated from a fluidic channel lined with EC's where monocytes are circulated. We hypothesized that TGF-β1 activates myofibroblast differentiation in the tendon hydrogel, stimulates tissue contraction and induces morphological changes in the ECs consistent with leaky and hemorrhaging vessels in acute tendon injuries.

Our experiments demonstrated the feasibility of modeling this tendon-vascular interface and the induction of fibrosis through treatment with TGF-β1 and similar results were observed by co-culturing with macrophages. Interestingly, the secretion of key cytokines such as CXCL10, IL-1β, and TNF-α were only increased in tri-culture conditions of TC, macrophages, and ECs in the *h*ToC. These cytokines evoke a range of inflammatory responses and CXCL10 in particular is a chemotactic for monocytes and induces their adherence to endothelial cells. Therefore, these results underscore the importance of including ECs in *in vitro* models of inflammatory fibrovascular tendon healing.

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. Therefore, the *h*ToC is designed as a tool to investigate fibrovascular scarring in tendon to identify key biological targets and as a drug screening platform for fibrosis.

Presentation: Oral

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Immune-competent 3D InSight™ tumor models for assessment of combinatorial biologics-based therapies

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Immunotherapy has revolutionized cancer treatment, but clinical success is still limited to a subset of patients, underlining the need for novel therapeutic targets. Available *ex vivo* platforms for drug screening show limitations due to only partial recapitulation of tumor-intrinsic features and of the interactions of tumors with stromal/immune cells. Here, we review our 3D InSight™ immuno-oncology model for study of the efficacy of immune-targeting agents on tumor infiltration and killing by peripheral blood mononuclear cells (PBMCs) and to acquire insights on the modulatory capacity of immune checkpoint inhibitors. The model system is implemented in InSphero Akura™ 384 plate format, which allows safe and accurate medium exchange with low residual volume, automated high content imaging and quantitation of fluorescent labels with low background & high sensitivity. The tumor viability and growth are evaluated with fluorescence measurements, the activation of immune cells is judged with cytokine profiling and the immune cell infiltration is assessed by histology analysis. Immune cells were cocultured with 3D tumor models and efficacy of anti-PD1 was assessed for 8 days by monitoring tumor viability based on fluorescence readout and y measuring the levels of characteristic cytokines including IFNγ, TNFα, IL-2 and GM-CSF in comparison to the respective control groups.

A temporal and spatial analysis of immune cell infiltration was performed using Yokogawa CellPathfinder software.

Presentation: Poster

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Mechanistic investigation of drug-induced liver toxicity using cellular causality assays in human 3D InSight™ liver microtissues

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Human 3D liver microtissues are more predictive than 2D hepatocyte cultures to screen for clinical drug-induced liver injury (DILI) and can also be used for investigation toxicology studies. Using specific treatment, the mechanisms of toxicity of Acetaminophen (APAP), Aflatoxin B1 and Trovafloxacin are successfully replicated *in vitro*. APAP produces a toxic phase I metabolite subsequently detoxified by cellular glutathione (GSH) *in vivo*. Correspondingly, APAP toxicity is potentiated by the inhibitor of GSH synthesis L-Buthionine Sulfoximine (BSO) in 3D liver microtissues. Trovafloxacin induces toxicity in inflamed liver *in vivo*. Correspondingly, 3D liver microtissues treated with lipopolysaccharides are more sensitive to the toxicity of trovafloxacin than untreated 3D liver microtissues. Finally, metabolism of Aflatoxin B1 by cytochrome P450 (CYP) 3A4 produces toxic reactive epoxides *in vivo*. Correspondingly, inhibition of CYP3A4 by the pan-specific CYP inhibitor 1-aminobenzotriazole (ABT-1) decreases Aflatoxin B1-mediated cytotoxicity in Human 3D liver microtissues, which suggests the formation of a reactive drug-metabolite.

Presentation: Poster

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Modelling biological mechanisms of a PNPLA3 polymorphism in *ex vivo* 3D human liver microtissues for NASH progression and drug efficacy

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Non-alcoholic steatohepatitis (NASH) is a progressive severe disease characterized by lipid accumulation, inflammation, and fibrosis in the liver. Single nucleotide polymorphisms (SNPs) at specific loci have revealed differential propensity to develop NASH. Among them, "GG" rs738409 located in patatin-like phospholipase domain-containing protein 3 (PNPLA3), is highly frequent (30-50%) and results in the I148Mamino acid change. PNPLA3 is a triacylglycerol lipase localized in lipid droplets but its function in the context of NASH is not fully understand and yet represents an interesting therapeutic target. The aim of this study was to investigate the effect of PNPLA3 I148M mutant on the development of NASH hallmarks in a 3D human *ex vivo* culture model.

Presentation: Poster

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Development of a novel transwell-based primary proximal tubule MPS model (aProximate MPS Flo™)

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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 an upsurge in physiological relevant microphysiological systems of the PT. Here we present our patented aProximate-MPS-Flo™ human PT platform, in which primary human PT cells seeded onto Transwell filter supports are subject to apical fluidic media flow and a shear stress of between 0.1-2 dynes/cm^2 in a 24 or 96 well filter holder The aim was to engineer an improved differentiation of cells, whilst retaining the utility and high throughput of a standard filter design. Under fluidic flow conditions, human primary PT cells formed monolayers with a Transepithelial Electrical Resistance (TEER) of between 60-90 Ωcm^2, similar to static culture conditions. Lucifer yellow (Papp 15.6 ± 06 cm/sec, n = 6) or 14C-mannitol (Papp 14.4 ± 0.3 cm/sec, n = 6) under flow conditions gave very similar results barrier function measured under static conditions. At the mRNA level OAT1, OAT3, OCT2 MATE1, MDR1, megalin and cubulin. Expression levels were elevated at least 5-fold compared to cell grown under static conditions. Flow also induced a significant increase in the number of primary cilia. The flux of 14C-creatinine in monolayers exposed to flow, the magnitude of net secretion was > 3 fold that of static cultures (J_{ab} 203.1 ± 12.6 pmole/cm^2/h, J_{ba} 398.2 ± 19.1, J_{net} 189.3 ± 14.6, $P < 0.001$, n = 6).In cells exposed to cisplatin for 48 hours, the key biomarkers; NGAL, KIM1 and clusterin were all significantly elevated ($P < 0.1$, n = 3) in monolayers under flow compared with static cultures.

This dataset, suggests that growing human PT cells on Transwell filter supports with media flow across the apical membrane, significantly improves phenotype and function and has significant benefit to the utility and near-physiology of the model.

Presentation: Poster

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Identification of variation factors for the development of assays for MPS

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The development of MPS (Micro Physiological System) has been progressing as an evaluation system that is expected to be utilized not only in drug development but also in life science in general. In particular, MPS is attracting a lot of attention as a cell-based assay with high extrapolability to humans that reflects physiological phenomena, such as "perfusion of culture medium" and "parallel culture of multiple organ cells," which are difficult to achieve with conventional culture dishes. On the other hand, the knowledge of culture technology using MPS is still in the process of accumulation, and there are still various issues to be solved. Under these circumstances, in Japan, cell developers, device developers, and users (pharmas) have been meeting since 2017 as part of the national project (AMED) to discuss device manufacturing technology, organ cell manufacturing technology, organ cell quality as MPS, and "standardization as MPS".

As data accumulation based on assay standardization strategy, we developed the sorting process for establishing assay methods. The most important thing is to clarify the test purpose and Context of Use (COU) in establishing the assay system. After that, it is easy to understand the requirements for the MPS by organizing the requirements for the MPS and breaking them down into requirements for the "cells" and "devices" that are the components of the MPS. In addition, it is desirable to optimize the assay design by accumulating actual data using cells and other materials for the MPS for which specifications have been tentatively determined, and finally to proceed to verification of eligibility and validation.

In this presentation, as the first step of the standardization of MPS, we will discuss the strategic data accumulation that is considered necessary for the development of assays using MPS.

Presentation: Oral

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Circadian physiology in microphysiological systems

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Disturbed circadian rhythm is implicated in chronic diseases, such as metabolic syndrome, fibrosis and cancer. The prevailing biomedical research paradigm emphasizing rodent and *in vitro* studies, limits or distorts the influence of circadian biology in conventional preclinical models. These limitations currently also extend to microphysiological systems (MPS).

Our interest in circadian cell physiology has been driven by proof of concept data implicating casein kinase 1delta (CK1δ) in fibrosis. The principal physiological function of CK1δ is phosphorylation of period proteins which regulates their level and nuclear localization thereby controlling the amplitude and phase of the cellular clock.

Transforming growth factor-β (TGF-β) is considered to be a master regulator of inflammation and fibrosis. We identified that CK1δ is involved in the signaling of TGF-β induced fibrosis. The absence of circadian cues for cells in culture means that the cells are either arrhythmic or asynchronously rhythmic. Thus, the pharmacology of CK1δ inhibitors may not be faithfully represented in conventional cell culture. We therefore decided to design, build and validate a platform technology for entraining circadian rhythm in cell culture and MPS systems.

Here we describe the operation of a rotary planar multiplexed microfluidic (RPM^2) pump which allows parallel perfusion of 24 and 96 multiwell plates. The RPM^2 has been used to entrain circadian rhythm by enabling the delivery of a daily cortisol pulse in continuously perfused, open cell culture systems in 24 and 96 well plates, but may also be applied to closed systems in an aspiration mode.

Reference

Gao, X., Cheng, T., Wu, Y. et al. (2022). Comprehensive multiplexed superfusion system enables physiological emulation in cell culture: Exemplification by persistent circadian entrainment. *Lab Chip 22*, 1137-1148.

Presentation: Oral

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Astronaut-on-a-chip: Human, multi-organ platform for assessing extended effects of cosmic radiation

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Cosmic radiation is one of the most serious risks encountered during long missions to the Moon and Mars, requiring effective measures for radiation protection. While animal models have contributed to our understanding of radiation damage and radiation protection, *in vitro* models consisting of engineered human tissues provide unparalleled physiological mimicry to humans exposed to galactic cosmic rays (GCR). Here, we report the use of a multi-organ-on-a-chip (OOC) platform in studies of systemic radiation consisting of engineered human tissue models of bone marrow (BM, site of hematopoiesis and acute radiation toxicity), cardiac muscle (CM, site of chronic radiation damage), liver (site of metabolism), and vasculature (barrier for transport of signals throughout system). To model the effects of GCR, tissue platforms were exposed to neutron radiation at Columbia's Radiological Research Accelerator Facility.

Using bioengineered human tissues derived from induced pluripotent stem cell or primary human sources, we show here: (1) extended cultures of a multi-OOC system containing CM, BM, liver, and vasculature after radiation exposures, (2) differential changes associated with acute versus protracted exposures, and (3) mitigation of radiation damage and longer-term effects using radioprotective drugs. We characterized the structural, functional, and molecular changes associated with longer-duration radiation exposures, and demonstrated an increased CD11b+ myeloid skewing of hematopoietic cells and decreased excitability of cardiac tissues, in response to 0.5 Gy acute neutron doses. This effect was even more significant in protracted exposures, of up to 0.5 Gy distributed in total over a period of 2 weeks. We also showed that administration of granulocyte colony stimulating factor (G-CSF) was able to subdue some of the hematopoietic injury in the engineered BM compartment. In future work, we aim to benchmark our engineered systems as closely as possible to data available on astronauts, animal studies, and accidental exposures to radiation on Earth.

Presentation: Poster

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Implementation of microphysiological system in a pharmaceutical company

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Pharmaceutical companies have expressed high expectations for microphysiological systems (MPS) in drug discovery and development (Morgan et al., 2018; Tetsuka et al., 2020). The first step in applying MPS to practical use is a respectful attempt to re-construct individual human organs by closely mimicking their structure, function, and cell composition. This reconstruction process differentiates MPS and organoid models from animal models, and requires innovative technologies in material science, microfabrication, tissue engineering, and developmental biology. Once a human MPS has been developed and well-characterized, it can be used for mechanistic elucidation of test articles from the viewpoint of drug disposition and safety when "unexpected" events are observed in clinical trials. Indeed, there seem to be many cases in which animal models used in preclinical studies do not fully cover the fate of drug candidates in humans because of species differences.

The next stage of interest from pharmaceutical companies may be to expand the use of these models/concepts to preclinical evaluation of the efficacy of test articles. For this, MPS must be further developed to accept pathophysiological cues into the models, a concept referred to as disease modeling. This will be achieved by either exposing the model to pathophysiological stimuli, or by incorporating cells/specimens from patients into MPS. Ultimately, the efficacy of test articles will be investigated by monitoring recovery from disease phenotypes in such MPS, or prevention of disease progression. In this presentation, our experience in the implementation of MPS in drug discovery and development will be presented, together with a case of disease modeling (Vormann et al., 2022; this study was funded by the Astellas Pharma Inc).

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Presentation: Oral

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Volatile response of lung cells to infection using a microfluidic lung-on-a-chip

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Animal based experiments have been the standard for investigating how diseases and infections affect the human body. However, due to ethical concerns and species differences, there has been a shift towards cell based assays utilizing human derived cells. Conventional *in vitro* cell culture systems often result in cell morphologies and interactions that do not replicate the original morphology or functions of the cell. Microfluidics have arisen as a solution to replicating *in vivo* cell cultures *in vitro* due to their ability to mimic the *in vivo* microenvironment and maintain cellular morphology and function. Due to the ongoing coronavirus pandemic, mimicking the response of lung cells to disease has become an important issue. We present a microfluidic device that allows the study of epithelial lung cells in response to viral infection. Using an electronic nose device (E-Nose), we were able to measure the volatile organic compounds (VOCs) that the cells produced in order to understand the cellular response to disease on a molecular level. By interrogating specific pathways, our results show, for the first time, the biological mechanisms that participate in the generation of the VOCs. The lung microphysical system provided a distinct advantage in terms of the cellular metabolites to air volume ratio, which enhanced the sensitivity and specificity. Our work will lead to more objective non-invasive biomarkers of infection and the subsequent point-of-care devices for rapid deployment. Furthermore, by utilizing a microfluidics based experimental setup, we are reducing the use of animal studies and producing results that preserve human physiology which facilitates the translation to clinical use.

Presentation: Poster

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Standalone microphysiological system with precise oxygen control for intestinal-microbial interactions

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The intestinal environment is unique because it supports the epithelial cells under a normal oxygen environment and the microbiota under a hypoxic environment (Albenberg et al., 2014). A major limitation of recent studies in modeling the hypoxic environment for the gut-bacteria interactions is that the oxygenation level of the epithelial cells is not known, raising the question whether the cells are hypoxic or not. As reported in previous studies, the hypoxic environment causes the intestinal dysfunctions (Xu et al., 1999; Zeitouni et al., 2016). We report a standalone intestinal microphysiological system that formed a partitioned oxygen environment to achieve coculture of intestinal epithelial in normoxic environment and anaerobic bacterial cells in hypoxic environment. The innovation was that the design was guided by a mathematical model of oxygen which considered diffusion and oxygen consumed by the intestinal cells, and supported by experimental studies.

We cocultured the intestinal cells with facultative bacteria, *E. coli* Nissle 1917 (ECN), and anaerobe, *Bifidobacterium adolescentis* (Bifido). We tested the mucus expressed by intestinal cells, barrier function of the intestine, and cytochrome P450 (CYP) enzymatic activity that related to the intestinal drug metabolism. Our result indicates both the ECN and Bifido not only induce the expression of mucus and protect the integration of intestinal barrier function, but to our surprise, increase the enzymatic activity by several fold as well. The results suggest the need to investigate further for drug pharmacokinetics and regulatory compliance and approval in drug discovery.

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Presentation: Oral

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Microphysiological systems to modeling basement membrane in the upper airway

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Understanding the impact of culture substrates on basement membrane regulation in *in vitro* models and the impact of mechanical and morphological cues on basement membrane function is crucial for upper airway physiological microenvironment models. In this research, we investigate the underlying physiology and model the microenvironment of the epithelial-mucosal lining of the upper airway by establishing an *in vitro* microphysiological model using Polycaprolactone (PCL) electrospun fibers. In this model, PCL fibers were used as a substrate for epithelial-fibroblast co-culture to mimic the mucosal layer and the basement membrane of the trachea and recapitulate the biophysical milieu of the native tissue. The electrospun fibers mimicked the architecture of the native basement membrane supported the proliferation of the human tracheal fibroblasts and supported the seeding of the human bronchial epithelial cells. The development of the epithelial mucosa and corresponding changes in mechanical stiffness in both membranes could be identified by *in situ* indentation. Coculture systems upregulate matrix remolding genes (mmp2, wnt, and sox2) while it downregulates fibrotic scarring in bmp and col1 signaling. In addition, the system matures in 14 days and the exposure to the air-liquid interface is immediately noticed in the TEER measurements. A mature basement membrane with functional mucosal membrane was produced using bilayer membrane; however, there is no significant difference observed in the coculture group with randomly oriented fibers compared to the aligned coculture group. The Random fibers showed trends of not encouraging a fibrotic phenotype, while the aligned fibers showed significantly greater basement membrane stiffness as well as an early increase in trans-epithelial resistance. A co-culture system was developed to model the tracheal mucosal microenvironment using fibers that mimicked the basement membrane, leading to a functional 3D *in vitro* model that mimicked tissue mechanics and transport function as well as cell-cell communication.

Presentation: Poster

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A BioStation CT based automated imaging system for a function-integrated microphysiological system platform

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Recently, microphysiological system (MPS) including organ-on-a-chip has been actively pursued in pharmacology. Microfluidic chips with two-layered channels equipped with porous membranes, such as the Lung-on-a-chip from Wyss institute, is widely used as MPS chips for various organs. However, bright field imaging of cultured cells on porous membranes poses difficulties associated with optical challenges. Another challenge of MPS is low throughput due to complicated handling. In this study, we have developed a function-integrated MPS platform integrated with two-layered MPS chips with a porous membrane and combined it with the BioStation CT, an automated imaging system developed by Nikon, to develop a cell-based assay system that enables high-throughput imaging.

The function-integrated MPS platform within the ANSI/SBS standard size consists of two two-layered microfluidic chips with porous membranes, micro-ring pumps for medium perfusion, a power supply system, and medium tanks. "BioStation CT for MPS" is equipped with the volume contrast (VC) method and a power supply system for the MPS platforms, which enables time-lapse observation of cultured cells on the porous membrane and fluorescence intensity in each channel in up to 15 MPS platforms.

In vitro nephrotoxicity tests were performed using our proposed system for functional evaluation. To construct an *in vitro* proximal tubular model, human renal proximal tubule epithelial model cells (RPTEC/TERT1 cells) were cultured on the membrane under flow condition in the chips. As a nephrotoxicity test, when RPTEC/TERT1 cells were exposed to different concentrations of cisplatin, changes in cell morphology were assessed by VC and changes in monolayer state were assessed by Lucifer Yellow leakage. The results showed that the automated time-lapse observation using this system could observe the dose-dependent decrease in cell number and increase in gaps of cisplatin even at low concentrations. Such an automated imaging system will be useful for the practical application of MPS.

Presentation: Poster

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A new multi-organ microfluidic device to recapitulate endocrine signaling *in vitro*: The LATTICE platform

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Current *in vitro* models used to study endocrine signaling and pathologies are unable to represent the complex tissue interactions found *in vivo*. To more accurately phenocopy human tissue-level cytokine and endocrine signaling *in vitro*, multiple 3D tissue models can be coupled together in a microfluidic platform. The organs of the female reproductive tract, including ovaries, fallopian tubes and uterus, are part of the highly regulated endocrine axis that is important for women's fertility and general health.

Our previous multi-organ microphysiological system (MPS) demonstrated our ability to recreate the menstrual cycle (Xiao et al., 2017) but had limitations such as high engineering complexity, costs, and lack of on-platform imaging. A new platform called LATTICE, was designed as a hormone-compatible, cost-effective, and user-friendly multi-organ MPS. It consists of two components, a computer controlled base station driving microfluidic flow and the LATTICE culture plates. Each plate has 8 tissue wells that are connected by rotary valve mechanisms and culture media is moved in microliter steps using customizable scripts. Flow rate is highly consistent and reproducible within a large range and using 36.35 µL steps ($\sigma = 0.31$) we can establish flow rates from 1 microliter/hour up to approximately 3.5 mL/hour. LATTICE is currently used to recreate some important tissue interactions that occur in polycystic ovarian syndrome (PCOS), a common multi-factorial endocrinopathy with endocrine and metabolic features in reproductive aged women.

Lastly, custom integration of the LATTICE platform with standard robotic handling systems allowed for the automatic imaging and tracking of dynamically cultured tissues. In summary, we have engineered and designed a simple and robust MPS system that will

enable us to study multi-organ endocrine inputs and their pathological disturbances.

Reference

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Presentation: Oral

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Suspended hydrogel membranes for tissue barriers-on-chips applications

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The role of tissue barriers is crucial in human physiological processes by delineating internal organs and regulating the intake and transport of oxygen, nutrients, and xenobiotics. In recent years, microfluidic technologies in the form of organs-on-chips and the implementation of new materials have produced significant advances mimicking the structure and function of biological barriers. Approaches using ECM hydrogels membranes to support the culture of cell barriers have shown a better emulation of normal *in-vivo* conditions than conventional membranes made of artificial polymers. However, the low-throughput and complicated fabrication processes limit the applications of these platforms. Therefore, we developed a novel method to integrate biological membranes in a high-throughput microfluidic device. First, we create an open microfluidic platform made of one main piece manufactured by 3D printing. After the final bonding of the printed part to an adhesive firm, we pattern the hydrogel solution by a simple loading-aspiration method. For this purpose, the device incorporates a suspended array of circular pores designed to hold the hydrogel solution in place. The fibrinogen solution is loaded through a channel that accesses and fills the pores from bottom to top. Subsequently, we aspirate the liquid using the same pipette, removing the hydrogel from the loading channel but the volume located in the pores stays suspended due to the action of the capillary barriers inside the suspended structure. We characterized the geometrical factors to successfully pattern the hydrogel using experimental and numerical simulation approaches. Additionally, we used this patterning method to form fibrin membranes and culture different types of cells on the top, bottom, and within the membrane. This patterning method offers a simple way to integrate biological ECM membranes to microfluidic devices for high-throughput tissue barriers-on-chips applications.

Presentation: Poster

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Optimization of the *in vitro* culture environment to maintain quiescent hepatic stellate cell for development of liver microphysiological system

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Hepatic stellate cells (HSCs) are non-parenchymal cells largely involved in fibrosis. Upon liver injury, HSCs are activated from a quiescent state and differentiate into myofibroblasts, secreting excessive extracellular matrix (ECM). When cultured *in vitro*, HSCs also spontaneously activate, rendering the development of liver microphysiological systems (MPS) difficult. In this work, we aim at optimizing culture conditions to maintain quiescent HSC *in vitro* through two parameters: ECM composition and stiffness. HSC maintained in quiescence can be further utilized into the development of liver MPS.

HSCs were differentiated from hiPSCs using a reported method. To optimize ECM composition, HSCs were cultured on type-I collagen or Matrigel. To optimize stiffness of culture environment, HSCs were cultured on dishes coated with a thin layer of ECM, on dish coated with a thick gel layer of ECM or embedded within the gel layer. TGF was supplemented to stimulate activation of the HSCs as a positive control. Immunostaining and qPCR were done after 5 days of culture.

Immunostaining showed that expression of activation marker SMA was upregulated in stiffer environment, regardless of ECM composition. qPCR results quantitatively confirmed that the HSCs are more activated when cultured in stiffer environment. qPCR results also showed that HSCs supplemented with TGF express significantly higher SMA in nearly all culture conditions.

Our results suggested that a softer culture environment is advantageous for maintaining HSC quiescence *in vitro*. In addition, differentiated HSCs could be further activated, which allows us to construct a liver MPS with a large contrast after stimulation. In the future, inclusion of further components of MPS such as flow and shear stress as well as supplementation with compounds such as insulin will be further investigated.

Presentation: Poster

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Development of multi-niche bone marrow-on-a-chip by controlling mechanical properties and oxygen gradients of matrix

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Bone marrow is the soft, highly vascularized tissue found in the medullary cavities of the bones that serves as main hematopoietic center in our body. Hematopoietic stem cells (HSCs) produce not only the red blood cells circulating for energy and gas exchange but also leukocytes, monocytes, lymphocytes which have essential role for immune system. For that reason, it is important to understanding bone marrow structure and its function. There have been efforts to develop artificial systems that mimic bone marrow microenvironment. Replicating the bone marrow microenvironment requires three structural components: the endosteal niche, the central niche, and the perivascular niche. Furthermore, to design realistic bone marrow culture systems, oxygen gradient between microenvironments should be considered. However, current artificial bone marrow system cannot fully replicate those key characteristics of the bone marrow due to its complexity and difficulty in reconstitution. In this study, we developed a bone marrow-on-a-chip with three types of matrices. Each of these matrices has different components and mechanical properties similar to each component of the bone marrow microenvironment. We also arranged matrices and microfluidics to create oxygen gradients along the components of the chip. This novel chip system showed high culture efficiency and efficiently recapitulated the cell population and function of the bone marrow. This can be used promisingly as an *in vitro* test system for diagnosis, drug screening, or research of diseases in which the immune system affects pathological progression.

Presentation: Poster

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Design and preliminary characterization of a new liver-on-chip model as a valuable additional tool for assessing *in vitro* drug toxicity and metabolism in a similar physiological environment

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Characterizing metabolic and safety properties of new drug candidates is one of the major targets of preclinical development. Although the current approach of regulatory agencies (e.g., EMA, FDA) relies on *in vitro* and *in vivo* models to safely test drugs in clinical trial, there is a strong need to anticipate any potential liabilities and to improve the predictivity of clinical outcomes starting from the very early stage of drug development.

Amongst different causes of failure, liver metabolism leading to potential hepatotoxicity and drug-induced liver injury (DILI) is still considered one of the most critical causes of failure during the development phases, as well as drug withdrawal from the market.

It is therefore essential to establish new *in vitro* tools able to reproduce the microarchitecture and physiology of the liver, to mimic the liver microenvironment.

To achieve this goal, a new liver-on-chip was designed, allowing a co-culture of hepatic and endothelial cells for assessing drug toxicity and metabolism in a similar physiological environment. This liver-on-chip has been conceived to be easily used and implemented in laboratory conditions thanks to a flow actuated by a simple rocker platform. Moreover, this pumpless system has been characterized in terms of basic functionality (e.g., albumin production, urea synthesis) and its suitability to reproduce the first-pass metabolism will be evaluated in the future. This platform may provide a useful model for liver toxicity studies and a potential value to better understand the metabolic process of drugs during their development steps.

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Presentation: Poster

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Complex *in vitro* models representing the pulmonary system

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In the past standard *in vitro* models including those representing the pulmonary system were based on single cell-type based models cultured under submerged conditions. In recent years complex models using more than one cell type in a 3D orientation have become more and more common. Models relevant for the lung have become commercially available and represent the whole respiratory system from the nasal cavity all the way to the alveolar barrier. Available models can be based on primary cells or cell lines with the inherent advantages and limitations of the two different approaches. Endpoints studied in the various models range from irritation, inflammation, respiratory sensitization, to carcinogenesis.

Such complex models need careful characterization of the properties from the individual cell types used for their assembly to the behavior of the co-cultured cells before and upon exposure. Most lung cells can be cultured at the air-liquid interphase (ALI). ALI culture mimics the *in vivo* situation in the lung. Changed properties and behavior of co-cultured cells have been shown under such conditions. Furthermore, the similarities and discrepancies of the 3D-models and the human *in vivo* tissue needs to be understood.

ALI culture allows to study effects of (nano)particles without any interference from a protein corona which would form under submerged conditions. Effects have been described to be different for submerged versus ALI culture conditions following exposure to chemicals.

Overall, models should be as complex as necessary to mimic physiological responses and as simple as possible.

Presentation: Oral

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How to best guide the characterization of a thyroid-liver chip: The relevance of combining pathological and metabolic readouts

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Microfluidic co-cultures of complex 3D organ models are considered as highly relevant for assessing the risk of potentially new therapeutics and agrochemicals. Along the establishment and characterization process of a 3D thyroid-liver co-culture for the rat and human, this presentation will discuss the importance of mimicking tissue structures in microphysiological systems (MPS) to reproduce morphological and metabolic readouts from animal studies.

The smallest functional subunit of the thyroid gland is presented by spherically shaped follicles. A microscopic analysis of different tested thyroid models, derived from primary tissue, was considered to be crucial to predict the functionality of the organ models. Only those rebuilding the follicular architecture were able to synthesize the thyroid hormones *in vitro*. Furthermore, changes in the morphology enabled to distinguish between thyroid-stimulating hormone-treated and non-treated follicles which was reflected on metabolic level when the amount of secreted thyroid hormones was compared. Lastly, the rat and human models were qualified to detect chemical-mediated thyroid hormone inhibitions in response to the TPO inhibitor methimazole.

To allow an interplay between the thyroid and liver organ models, the presence of the hepatocellular polarity is key to successfully mimic the hepatic thyroid hormone metabolism. By immunofluorescence staining we demonstrated the expression of the monocarboxylate transporter 8 (thyroid hormone transporter) and a bile canaliculi network (required for the thyroid hormone metabolite efflux) in the liver spheroid models. Given this functional structure and the activity of thyroxine-specific UDP-glucuronyltransferases and sulfotransferases, the glucuronidated and sulfated-thyroxine metabolites gT4 and sT4 were actively formed and secreted by the liver spheroids. These catabolites finally served as functional metabolic markers to evaluate chemical-induced changes of the thyroid hormone metabolism.

Furthermore, the presentation will outline methodical hurdles and give an overview of the co-culture approach using a commercially available 2-Organ-Chip.

Presentation: Oral

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Dynamic lung inhalation-on-chip: A triple co-culture cellular platform to predict toxicity of aerosolized irritants

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Persistent injury to the alveolar epithelium induced by inhaled toxicants is a hallmark event in the pathogenesis of emphysema, a severe manifestation of chronic obstructive pulmonary disease (COPD). Inhalation is a major route for exposure where the pulmonary epithelium serves as the portal of entry to the systemic circuit for airborne irritants. Limited alveolar *in vitro* models are currently available highlighting an urgent need to develop alternative "new approach methodology" which are able to reproduce the alveolar microenvironment (breathing dynamics) and inhalation mechanisms.

To this end, we used primary-derived immortalized human alveolar epithelial cells (AXiAECs) along with THP-1 derived macrophages and human endothelial cells in the AXLung-on-Chip System (AlveoliX AG). We subjected the cells to cyclic stretch and air-liquid interface (ALI) conditions on the chip. To recreate occupational inhalation, we exposed the lung epithelial-barrier to varying doses of nanoparticles (ZnO, TiO_2, SiO_2) and a toxic compound (PHMG) using the Cloud-based aerosol exposure chamber (Cloud AX12, Vitrocell GmbH).

Our results demonstrated a stable alveolar barrier formation represented by distinct tight junction protein expression and gradual increase in transepithelial electrical resistance. The toxic compound PHMG was used for toxicity studies. Epithelial-endothelial co-culture with differentiated THP-1 macrophages resulted in an escalated inflammatory response upon treatment with PHMG, starting from 4 hours. Furthermore, decreased barrier integrity (~3 fold) along with decreased expression of COPD-distinct markers (Muc1, CD206) and increased pro-inflammatory gene expression (IFNγ, IL1-α and IL1-β) were observed in cells nebulized with PHMG, in physiologically relevant conditions (ALI-breathing). Similarly, aerosolized nanoparticles induced significant toxicity as measured by cell viability and ROS release in ALI-breathing cells compared to submerged-static conditions.

In summary, our findings establish this breathing lung-on-chip system combined with the Cloud AX12 exposure module as a promising and versatile tool to study emphysema, inhalation toxicology and other acute respiratory disorders.

Presentation: Oral

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Efficient generation of functional liver organoid from human iPS cells-derived CPM$^+$ liver progenitor cells using an oxygen-permeable microwell structure

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Through recent technological advances in bioengineering, human induced pluripotent stem cells (hiPSCs) appear as a promising alternative source for the generation of liver organoids for disease modeling or drug screening. Yet issues remain in terms of tissue maturity and cost of the induction due to the use of costly growth factors. Therefore, an efficient method for the generation of functional liver organoid and the enhancement of tissue maturity is needed.

Here, we utilized the Carboxypeptidase M (CPM) as a cell-surface marker to sort liver progenitor cells (LPCs) derived from hiPSCs. Afterward, we generated homogenic hepatic organoid with those cells by using a microwell structure and targeted the improvement of maturation through direct oxygenation of the culture with a PDMS membrane. Culture optimization of the liver organoid was evaluated in terms of morphological analysis, gene expression profile, and functionality such as albumin synthesis and CYP3A4 activity assay. The formation of a potential bile canaliculi network was also observed through immunostaining and a CLF uptake assay.

Liver organoids were successfully formed from hiPSCs-derived CPM$^+$ LPCs. The resulting tissue consisted of hepatocytes and cholangiocytes as indicated by the gene expression of albumin and cytokeratin 7, respectively. It was found that in high-density culture ($1x10^6$ cells/cm^2) using microwells with a diameter size of 326 μm, cells organized as dense spheroid with the highest albumin secretion (~58 μg/$1x10^6$ cells/cm^2) and the highest CYP3A4 activity. Results in terms of albumin gene expression were comparable to primary hepatocyte. Accumulation of CLF in the cyst area of the organoid was also observed and supported by the expression of the major bile transporter MRP2 and BSEP. The present method has allowed for the generation of highly mature liver organoids composed of different cell populations. Inclusion with parameters such as perfusion in microphysiological systems may further improve the results observed.

Presentation: Poster

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uHeart, a beating heart-on-chip cardiac model for predicting drug-induced functional cardiotoxicity

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In the drug development process, functional cardiotoxicity and potential pro-arrhythmic effects of drug candidates represent one of the major concerns. Generating relevant *in-vitro* cardiac models better representing the human heart with integrated fit-to-purpose assays, has been recognized as an urgent need.

Here we present uHeart[Visone_Biofabrication,2021], a human functional 3D cardiac microtissue developed in a beating heart-on-chip platform integrating two technologies: uBeat®, providing a relevant mechanical stimulation to 3D microtissues[Marsano_Lab-Chip,2016] and μECG, to record on-line the cardiac electrophysiology. The model has been qualified to assess the cardiotoxic effects of known-compounds.

Human induced-pluripotent-stem-cell derived cardiomyocytes were embedded in fibrin hydrogels with supportive fibroblasts ($100\text{-}125 \cdot 10^6$ cells/mL, 75%-25% ratio) and were mechanically trained (i.e., 10% uniaxial strain at 1 Hz) for 7 days. A cardiotoxicity screening campaign was performed by administering 11 drugs at increasing doses. Relevant field potential (FP) changes (e.g., beating parameters and arrhythmic events) were analyzed by a post processing MATLAB-based algorithm.

After one week of culture, the human cardiac microtissues spontaneously beat as a syncytium generating a clear cardiac FP signal, whose patterns were automatically recognized by the algorithm. The microtissues showed a beating period of 1.9 ± 0.7 s and a FP duration (FPD) of 0.69 ± 0.25 s. Drug screening results evidenced that DMSO (vehicle) and Aspirin (negative control) did not alter cardiac electrophysiology. Conversely, Ikr blockers (e.g., Dofetilide, Quinidine) prolonged the FPD at concentration near the Cmax, while ICaL blockers (e.g., Verapamil, Nifedipine) shorten it already at 5-50nM. Mexiletine, blocking INa, statistically decreased the FP amplitude at 10 μM. Both Terfenadine and Dofetilide elicited arrhythmic events, matching FDA labels indications. Overall, the system showed 83.3% sensitivity, 100% specificity and 91.6% accuracy in detecting FPD prolongation.

uHeart predicts drug cardiotoxic effects and together with the automatic algorithm for the FP analyses represents a valuable tool to perform cardiotoxicity screening.

Presentation: Poster

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Human endometrium-on-a-chip model of the secretory phase for substance exposure studies

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Introduction: The endometrium is a highly dynamic tissue where classical cell culture-based test methods fail to correctly reproduce a relevant phenotype and treatment response. Biology-inspired micro-physiological systems, such as those based on TissUse's HUMIMIC Chip platform, might more closely resemble organ complexity and crosstalk to mimic the endometrial secretory phase. Our human 3D endometrial model (EM) is composed of immortalized endometrial stromal cells (THESC), an endometrial epithelial cell line (EM42) and primary human uterine endothelial cells (HUtMECs) and has been optimized to undergo physiological changes upon addition of the steroid hormones estrogen and progesterone.

Methods: The 3D EM, initially generated in a static culture system, was integrated into the HUMIMIC Chip2 96-well, whose microfluidic channels were seeded with HUtMECs, and cultured for up to 10 days inside the chip under circulatory perfusion conditions. Viability and homeostasis were monitored by LDH release and metabolic profiling throughout the length of the assay. Furthermore, integrity and functionality of the 3D EM were additionally evaluated by immunofluorescence (stromal marker: vimentin; endothelial markers: CD31 and vWF; epithelial marker: cytokeratin), and following the addition of steroid hormones, decidualisation markers such as IGFPB1, PRL and FKBP5 were confirmed by qPCR and ELISA.

Results: Immunohistochemical analysis and ELISA measurements of decidual protein secretion (IGFBP1) showed cellular transformation of the 3D EM together with an increased protein secretion, respectively. This steroid hormone-induced decidualisation process could be partially inhibited by co-treatment with ulipristal acetate, a selective progesterone receptor modulator. Moreover, CalceinAM viability staining together with endothelial markers´ staining demonstrated that HUtMECs were viable and functional throughout the course of the chip culture.

Conclusions: The implementation of the model in the HUMIMIC Chip2 96-well allows to elucidate treatment effects on (patho-) physiological parameters in a complex tissue environment and could be used in future to model the complete menstrual cycle.

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Scaffold free human adipose spheroids model: Phenotype dependent inflammatory response

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The predisposition to weight increase, the onset of diabetes and cardiovascular diseases are related to a wide filed of metabolic disorders, where environmental factors, phenotypical/genotypical aspects affect fatty acid accumulation and adipose tissue homeostasis leading to a pro-inflammatory status.

The growing interest in these issues paved the way to the development of adipose tissue models able to mimic the unbalanced homeostasis of adipose tissue in metabolic disorders. 3D scaffold-free adipose spheroids were produced by hanging drops technology using primary pre-adipocytes at early passages isolated from overweighted male and female donor (BMI value > 25). In the tridimensional configuration, pre-adipocytes can fully differentiate in mature adipose tissue after 15 days of culture, preserving their native cell-cell and cell-matrix interactions and providing a more reliable micro-physiological system able to mirror the main features related to a specific phenotypic profile in a 3D geometry.

To simulate the metabolic disorder associated to a pro-inflammatory status, spheroids were stressed with increasing concentrations of LPS (10, 20 and 100 ng/mL) during 24 h and immediately after pro-inflammatory stimulus, metabolic activity by the ATP assay, IL-6 gene expression by RT qPCR, IL-6 release in the culture media by ELISA assay were quantified.

For both spheroids' series, LPS exposures did not affect the metabolism, but the stimulation triggered a prompt pro-inflammatory response in a dose-dependent manner with different responsiveness according to the native donor's phenotype (different for age and gender). Female donor showed a greater susceptibility to inflammation inductor, with a progressive increase of IL-6 gene expression and release compared to male donor and to untreated control.

The results highlight that the establishment of a 3D micro-environment provides the optimal physiological guidance for primary cells polarization and aggregation, preserving their native donor-dependent phenotype useful for investigating causes of specific susceptibility to metabolic disorders and personalized therapies development.

Presentation: Poster

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Blood-brain barrier on-a-chip for high throughput barrier and transport studies

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Drug delivery across the blood-brain barrier (BBB) represents a significant clinical challenge, largely due to its low permeability, high selectivity, and the presence of efflux transporters. As such, *in vitro* BBB models are widely used for prediction of permeability and toxicity of compounds. These models are typically achieved via culture of brain endothelial cells on permeable membrane inserts such as Transwell. However, such models are limited in physiological relevance due to their 2D nature, lack of extracellular matrix, presence of an artificial membrane, and absence of perfusion. Therefore, microfluidic models are becoming increasingly adopted. The OrganoPlate is a microfluidic platform enabling perfused, 3D co-culture of up to 96 tissues in a membrane free manner. It allows growth of tubular endothelial structures and is based on a 384-well plate. To create a BBB model, brain endothelial cells are seeded against a collagen-I extracellular matrix. Under perfusion, endothelia form confluent tubule structures, accessible from both apical and basolateral sides. The model comprises a perfused 3D microvessel of human brain microvascular endothelial cells (hBMECs), with the option to include other cell types such as astrocytes and pericytes. It shows expression of relevant phenotypic and junctional proteins, transporters, and receptors. Using fluorescent assays, we demonstrate low permeability of primary hBMECs to sodium fluorescein, as well as functional P-glycoprotein and GLUT1 activity. Barrier and transport readouts can be performed directly in-plate without the need for sampling. Time-dependent barrier disruption by toxic compounds is measured in 40 cultures in parallel with TEER (OrganoTEER). This BBB model in serves as a versatile model for high-throughput applications including toxicity and transport studies.

Presentation: Poster

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Modeling ischemic stroke in a triculture 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 three-fold 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.

Presentation: Oral

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A PBPK-compliant human intestine-liver-brain-kidney chip for QIVIVE in drug development

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Microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue- and organ-like functions. However, establishing complex human *in vitro* ADME models involving co-culture of key organs to mimic physiologically based pharmacokinetic (PBPK) distribution behavior still present a challenge. 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 and first-pass metabolism, liver microtissues for 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. The setup was repeatedly exposed to Haloperidol, an antipsychotic medication and to Carbamazepine, a tricyclic compound with anticonvulsant properties through different routes. Results on direct as well as metabolite induced effects on organ-specific levels will be presented. Subsequently this data formed the basis for the development of an *in silico* PBPK model for compound prediction.

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Presentation: Oral

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Optimization of an angiogenesis-on-chip model and its implementation into drug discovery

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Background: Angiogenesis is related to various diseases, such as cancer, and is initiated by a gradient in angiogenic factors. Angiogenic sprouting can be mimicked in microphysiological systems (MPS) employing a concentration gradient in angiogenic factors. For implementation of such models by pharmaceutical use, traceability of the protocol and versatility of the cell sources are key factors. In this study, we optimized an earlier established angiogenesis-on-a-chip model (van Duinen et al., 2019) to a common endothelial cell source and ran assays in different research laboratories.

Method: Perfused human umbilical vein endothelial cells (HUVECs), cultured in an OrganoPlate® 3-Lane against a collagen I extracellular matrix, were exposed to sprouting mix (VEGF, S1P, and PMA) in the opposite channel to induce angiogenesis. Sunitinib, an anti-angiogenic modulator, was added to both channels in the presence of the sprouting mix. Sprout formation and possible inhibition by sunitinib was quantified after exposure for 2 days, using phase contrast imaging and confocal imaging after Calcein-AM and Hoechst staining. In combination with several developed quantitative image analysis, differences in sprout distance and area were determined. The protocols established at MIMETAS were transferred to Astellas Tsukuba Research Center (Ibaraki, Japan), who then performed the same experiments using the same lot of HUVECs.

Result: The optimized angiogenesis-on-a-chip model showed sprout formation upon exposure to the sprouting mix. Sunitinib treatment showed a dose-dependent inhibition on sprout formation in the model. After the establishment of this protocol at MIMETAS, Astellas ran this assay according to the established protocol and confirmed sprout formation and sprout inhibition by sunitinib. These results highlight the versatility of the optimized model and assay. The angiogenesis-on-a-chip model showed to be a promising tool in studying anti-angiogenic modulators, which is a key focus in drug discovery.

This study was funded by Astellas Pharma Inc.

Reference

van Duinen, V. et al. (2019). *Angiogenesis 22*, 157-165.

Presentation: Poster

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Automation and validation of the OrganoPlate LiverTox for hepatotoxicity detection

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Drug-induced liver injury is one of the leading causes of market withdrawal in the pharmaceutical industry and poses a serious health risk to affected patients. Here, we developed a 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. 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. Characterization of the model revealed hepatocyte function including CYP3A4 activity and albumin production for up to 14 days. Fetal hepatocyte marker alpha-fetoprotein (AFP) declined over the 14 day culture, supporting iHep maturation. Assay validation studies using troglitazone as a positive hepatotoxic control 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. Together, the OrganoPlate LiverTox is a promising platform for hepatotoxicity detection and has the potential to be used in a high throughput screening capacity.

Presentation: Poster

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AlvireX – An advanced drug screening platform for respiratory viruses

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Respiratory viruses pose a constant threat to public health and can potentially cause severe disease such as coronavirus disease 2019 (COVID-19). The causing agent, Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), exploits angiotensin converting enzyme 2 (ACE2) and host proteases for cell entry. In the distal lung, SARS-CoV-2 infection interferes with normal function of type I and II alveolar epithelial cells (AEC), which may result in the breakdown of the air-blood barrier and massive inflammation. The extreme difficulty to model the alveoli *in vitro* and *in vivo* has limited studies on severe COVID-19. Eventually, human-derived 3D *in vitro* models, such as organoids and organ-on-chip technology, allow disease modelling and drug testing in a human-relevant setting.

We aim to develop AlvireX, a drug screening pipeline for COVID-19. AlvireX combines drug pre-screens in alveolar organoids and subsequent validation in a lung-on-chip model (LOC) to shortlist candidate drugs.

Alveolar organoids and an alveolar epithelial cell line for on-chip applications have been generated from primary human AEC. Alveolar markers and host factors were analyzed by fluorescence imaging and gene expression. Finally, AEC were infected with SARS-CoV-2 and monitored for virus production and barrier function by virus titration, immunofluorescence imaging and transepithelial electrical resistance (TEER) measurement.

We confirmed the alveolar-like phenotype (HTII-280, SP-C, HTI-56, AQP5) of organoids and immortalized AEC. Moreover, they expressed relevant host factors (ACE2, TMPRSS2), which were further upregulated in air-liquid interface. AEC were susceptible to SARS-CoV-2 and released viral progeny from the apical side. Moreover, TEER was significantly decreased in infected cultures indicating virus-mediated barrier breakdown.

Recapitulating aspects of severe COVID-19 we employ our models for toxicity and efficacy screening of candidate drugs against COVID-19. We expect that AlvireX will expedite drug development against current and emerging respiratory viruses and provide valuable insights into host-pathogen interactions in the distal lung.

Presentation: Poster

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Mesenchymal stem/stromal cells of different origin affect vascular network phenotype in a perfused microfluidic chip

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Generating a three-dimensional and perfusable vasculature is vital for reliable tissue modelling. Essentially, vascular network formation involves two cell types: vessel-forming endothelial cells (ECs) and vessel-supporting pericytes. During vascular network assembly, tissue-specific mesenchymal stromal cells, MSCs, are known to function as pericytic progenitor cells supporting the endothelial cells during vessel formation. Still, it is unclear how different MSCs as pericytes affect the vascular network phenotype and functionality.

Here, we compared the pericytic functionality of human MSCs, bone marrow-derived stem/stromal cells (BMSCs) and adipose stem/stromal cells (ASCs).

We generated 3D vascular cocultures of ECs with BMSCs or ASCs. We embedded the cells in fibrin suspension within a commercially available microfluidic chip (AIM Biotech). Gravity-based interstitial flow across the hydrogel area was characterized in terms of flow rate and duration. After 6 days of culture, the formed vascular perfusability and maturity was examined. Also, the vasculature area, vessel length and diameter were quantified in both cocultures. RT-qPCR was performed to compare the angiogenic gene expression of EC-BMSCs and EC-ASCs.

Both cocultures resulted in perfusable, 3D vascular networks. Both networks were enfolded by collagen IV demonstrating the presence of basement membrane and CD144 indicating intact endothelial barrier. During vessel formation, the maximal interstitial flow velocity resulted in a physiologically relevant shear stress (6.5 mPa) across the hydrogel area in both co-cultures. After network formation, BMSC-supported vasculature was found to be fully perfusable with larger vessel area whereas ASC-supported vasculature was found to be partially perfusable. In addition, BMSC-supported vasculature had significantly higher expression in genes involved with vascular maturation.

To conclude, we demonstrated that human MSCs of different origin affect the forming vasculature resulting in distinct vascular network phenotypes. These findings allow us to generate tissue-specific vasculature for the currently developed bone and adipose tissue *in vitro* models.

Presentation: Poster

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A microfluidic thyroid-liver platform to enable cross-species comparison of mechanisms of thyroid toxicity in rats and humans

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Toxicity studies in rats are a mandatory requirement for the market authorization of new molecular entities (NMEs). However, rats are very susceptible to perturbations of the thyroid homeostasis and frequently develop thyroid alterations, such as adenomas, upon chronic exposure. Due to the fundamental role of thyroid hormones (THs) across species, evidence of interference of NMEs with the thyroid homeostasis of rats understandably raises concerns for human safety. Thyroid disrupting chemicals (TDCs) interact either directly with the thyroid gland or exert effects indirectly, e.g., through induction of the hepatic TH catabolism. Rats and humans differ in key parameters of thyroid homeostasis, e.g., in the half-life of circulating THs. Moreover, chemicals may induce species-specific liver enzyme activation which, altogether, impede the translation of thyroid findings to humans. To this end, we have established *in vitro* thyroid-liver co-culture models for rat and human, utilizing TissUse's HUMIMIC platform. Their application is meant to identify TDCs and compare sensitivities across species on organ-level functions *in vitro*. The thyroid compartment, containing isolated thyroid follicles embedded in extracellular matrix (ECM), displayed a stable follicular architecture under both static and microfluidic culture conditions. Its stimulation with thyrotropin (TSH) induced TH release which diminished upon treatment with the thyroperoxidase (TPO) inhibitor methimazole. The liver compartment, consisting of ECM-embedded liver spheroids, maintained key hepatocellular features such as bile canaliculi formation, albumin secretion, and a constitutive TH catabolism which was inducible by nuclear xenobiotic receptor activators like beta-naphthoflavone. Both compartments were successfully combined in microfluidic thyroid-liver co-culture models and remained viable and functional for at least 14 days. Further optimization is required to achieve stable long-term functionality of both models. However, the current study already demonstrated the feasibility to recapitulate direct and liver-mediated indirect chemical disruption of thyroid homeostasis on organ-level functions in a single *in vitro* assay.

Presentation: Oral

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Patient-specific organ-on-chip model of the human cervix and cervical cancer

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While 80% of sexually active people have an infection with the Human Papilloma Virus (HPV) at least once in their lifetime, only few of them will notice as the infection clears itself in most cases. However, if the infection persists, a cervical intrepithelial neoplasia (CIN) can occur and lead to cervical cancer, which is the 4th most common cancer within women, leading to 270,000 deaths worldwide. To gain mechanistic insights as well as develop novel therapeutic options, human-relevant, physiological *in vitro* models of cervical tissue and CINs are urgently required.

Methods: Cells were isolated from human cervical tissue biopsies and integrated as co- or tri-cultures in a Cervix-on-Chip (CXoC) mimicking physiological architecture of the cervical tissue. For disease modelling, CIN or cancerous clusters in the stroma were emulated with human squamous cervical cancer cells. The hybrid-MPS was fabricated from microstructured thermoplastic elastomers, polymethylmethacrylate and polycarbonate. Cervical tissue was engineered via dynamic culture at the air-liquid interface and cell types and status comprehensively characterized via on-chip staining or histological analysis.

Results: The open-top design of the CXoC allows static and dynamic cultivation, in submersed or air-lifted condition, to meet the requirements of the differentiation protocol. The integration of elastic materials in the platform ensures system robustness without leakage for long-term cultivation. Patient-specific human primary cervical fibroblasts, keratinocytes and endothelial cells were integrated into the CXoC. While the endothelial cells line the micro-channels, mimicking blood vessels, the keratinocytes develop an epithelium on top of fibroblasts embedded in hydrogel. For disease modelling, aggregates of cervical cancer cells resembling infiltrating nests are integrated in the stroma.

In conclusion, we established healthy and diseased models of the human cervix in a microphysiological system that captures the 3D arrangement of several cell types, mimicking human (patho-)physiology.

Presentation: Poster

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Complex *in vitro* models in preclinical toxicologic pathology – histotechniques and examples

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Complex human-based *in vitro* models (CIMs) offer an unprecedented opportunity to support enhanced preclinical to clinical translation through high-quality preclinical mechanistic data and are contributing to the 3R principles: Reduce, Refine and Replace. However, developing robust high-throughput (HTP) histotechniques for this wide range of CIMs remains a great challenge due to differences in size, culture requirements and cell composition. We have developed five end-to-end HTP histotechnique workflows for different CIMs ranging from three dimensional spheroids and organoids to microfluidic chips. Subsequent application of immunohistochemistry and multiplex immunofluorescence stainings demonstrate specific cell-marker expression as well as persistence of spatial information and endogenous cell labels. The application of these workflows enabled detailed model characterization at different timepoints. Overall, our developed histotechnique workflows will support accurate use of CIMs for the assessment of efficacy and toxicity in a reproducible, robust, sensitive and HTP manner in preclinical drug development.

Presentation: Oral

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Morphologic automated AI readout of *in vitro* experiments in preclinical drug development

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Developing robust and reproducible high-throughput (HTP) morphologic readouts for the wide range of complex *in vitro* models remains a great challenge in preclinical *in vitro* toxicity assessment. To address this challenge, we developed an innovative automated HTP artificial intelligence (AI) algorithm that detects single cell toxicity in Hematoxylin and Eosin (HE) 40x images of blood-brain barrier (BBB) organoids. We generated two types of ground (GT) truth data that were used for the nuclei segmentation and detection of apoptotic/necrotic nuclei in the HE scans (VIS, Visiopharm): Caspase-3 immunohistochemistry (IHC) detecting apoptotic nuclei was used as objective GT, while the subjective GT included evaluation of apoptotic/necrotic nuclei on HE slides by three veterinary board-certificated blinded pathologists on 40 randomly chosen BBB organoids. Pixel-based accuracy for nuclei segmentation was almost perfect (F1 score = 0.98), while pixel-based accuracy for apoptotic detection (Caspase-3 IHC) showed moderate performance (precision P = 0.34, recall R = 0.77 and F1 score = 0.46) as not all putative apoptotic/necrotic nuclei were positive for Caspase-3 leading to low precision. Correspondingly, object-based accuracy (pathologists' evaluation) demonstrated an increase in precision (P = 0.64, R = 0.7, F1 = 0.65), indicating a morphological detection of apoptotic/necrotic nuclei by the AI algorithm. To validate the entire workflow, we treated BBB organoids with different concentrations of staurosporine to induce apoptosis/necrosis and compared the results with those from a standard low-magnification fluorescent caspase 3/7 assay. Our automated AI histomorphology readout was comparable to the standard assay, while further adding single cell and spatial resolution, digitalization, automation, HTP and reproducible components. Our developed label-free end-to-end digital readout will accelerate decision-enabling experiments in preclinical drug development and in the future, can be transferred to other organoid models.

Presentation: Poster

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Testicular *in vitro* models applicable in toxicology and biomedicine: Current status and future prospects

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Health problems due to distortion of male reproductive health and fertility (around 7% of men suffer from infertility) have increased in recent decades and become a concern for researchers and the general public. Pathophysiological conditions, such as exposure to environmental pollutants, drugs or chemotherapeutics, radiation or cancer, are widely discussed factors contributing to these adverse outcomes. Therefore, the testis is a priority organ for developing *in vitro* models to assess male reproductive health hazards of chemicals, investigating therapeutic interventions to male reproductive disorders or studying testicular pathologies. *In vitro* testicular models should reflect the critical aspects of male reproductive health, key (patho-)biological processes and mechanisms, including disruption of spermatogenesis and steroidogenesis, and cover crucial windows of susceptibility. Co-cultures of testicular cells and three-dimensional (3D) models better mimicking the complexity of the testicular cytoarchitecture and cytophysiology than two-dimensional (2D) monolayer single testicular type are powerful model systems. Therefore, recently, testicular cell-based co-cultures and 3D testicular models and organoids have started to garner interest. This presentation will describe the current status of testicular *in vitro* models applicable in toxicology and biomedicine. In addition, an outlook on existing applications and future directions in this field of research, including microphysiological systems, will be presented. We will also introduce a 3D *in vitro* model of murine prepubertal Leydig TM3 cells suitable for image-based screening of testicular toxicity and an *in vitro* co-culture model of murine Leydig TM3/Sertoli TM4 cells for monitoring cell morphology, behavior and function in testicular toxicity assessment.

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Presentation: Poster

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Development of a lymphoid organ-on-chip to evaluate CD4$^+$ T cell/B cell interactions

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Background: Secondary lymphoid organs provide the adequate microenvironment for the development of antigen (Ag)-specific immune responses. The tight collaboration between CD4$^+$ T cells and B cells in germinal centers 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. To address this issue, we developed an *in vitro* 3D model of human lymphoid organ using a two-compartment microfluidic chip.

Methods: CD4$^+$ T cell- and B cell-enriched PBMC were seeded in the top channel of Chip S-1® (Emulate™) lined with HUVEC endothelial cells. The bottom channel of the Chip was filled with an extracellular matrix (ECM) loaded with antigen that mimicked the lymphoid stroma. Immune cell migration and cluster formation were monitored by imaging, plasmablast differentiation by flow cytometry, and antibody secretion by a cell-based binding assay (S-flow).

Results: Using fluorescently labeled cells to monitor cellular dynamic in the Chips, we observed that B and CD4$^+$ T cells migrated from the top to the bottom channel in presence of superantigen in the matrix. Cellular migration was enhanced in the presence of fluid flow and of an endothelial barrier at the channel interface. Dynamic CD4$^+$ T cell/B cell interactions took place in the ECM, with large aggregates observed in the presence of superantigen. Chip perfusion with the SARS-CoV-2 spike protein resulted in the induction of Ag-specific recall responses from cells of volunteers with positive serology to the protein. B cell maturation into plasmablasts and concomitant CD4$^+$ T cell activation were documented by flow cytometry. For a subset of the volunteers tested, spike-specific IgG secretion was detected as early as Day 6 in chip effluent medium.

Conclusion: We developed a lymphoid organ-on-chip suitable for monitoring induced memory responses after infection or vaccination.

Presentation: Oral

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Characterization and ranking of PBMCs for drug safety assessment in immunocompetent human organ models

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Immunocompetent human *in vitro* models are emerging as the new frontier for preclinical safety assessment of novel immunomodulatory therapeutics. In this context, organ models are co-cultured with peripheral blood mononuclear cells (PBMCs). PBMCs are incorporated into the culture system to enable simulation of treatment-mediated activation of immune cells and subsequent immune-mediated organ injury.

The characteristics and composition of PBMCs vary from subject to subject and from batch to batch. The factors responsible for this variability are diverse, ranging from genetic and environmental factors, donor medication and stress level to factors during blood withdrawal, sample transport and sample preparation. Since this variability influences the response levels of immune cells to activation triggers such as immunomodulatory drugs, it is advisable to characterize PBMCs in an adequate way to control this variable in the *in vitro* evaluation of drug safety.

In order to generate a bank of characterized PBMCs, PBMCs were isolated internally from buffy coats or bought from external providers. PBMCs were activated *in vitro* using different stimulation reagents and analyzed by multiparametric flow cytometry with the aim to classify donors according to their immune activation profile. The 25-marker flow panel consisted of surface markers (lineage and activation markers) and intracellular markers and cytokines. Almost 40 parameters derived from the flow cytometry analysis were integrated to classify the donors on the basis of their activation status. A final score was calculated by adding up the position numbers of each donor for each parameter. Donors were then divided into three groups of high, mid and low responders.

In conclusion, the PBMC bank consisting of ~40 highly characterized donors allowed choosing the activation profile that best suited the assessment needs and using the same PBMC donors across different assays for *in vitro* safety packages. Furthermore, this approach will support the investigation of donor-to-donor variabilities.

Presentation: Poster

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In vitro organization of lymph node stromal networks: Impacts of matrix composition and interstitial fluid flow

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Lymph nodes (LNs) are highly organized immune organs found throughout the body that provide immunosurveillance and initiate the adaptive immune response. Developing microphysiological LN models is key toward replicating immunity *in vitro*. A network of fibroblastic reticular cells (FRCs) and lymphatic endothelial cells (LECs) guide immune cell trafficking within the LN using direct contact as well as secretion and positioning of key chemokines, CCL19 and CCL21. Toward the goal of developing a spatially organized LN-chip, the self-organization of FRCs and LECs *in vitro* was examined in three materials: a photocrosslinkable thiol-modified gelatin (GelSH) hydrogel, a combination photocrosslinkable hyaluronic acid (PhotoHA) – type I collagen hydrogel, and a type I collagen – fibrinogen hydrogel. The benefits of interstitial fluid flow (IFF) for LEC-FRC networks were examined using a transwell setup with pressure head induced IFF. Fluid flow through the networks was further quantified using magnetic resonance imaging (MRI) to track alterations in flow profiles. Enzyme linked immunoassays (ELISAs) were used to determine CCL19 and CCL21 secretion. LECs and FRCs are shown to form more extensive networks when co-cultured compared to either alone and show sensitivities toward matrix stiffness, composition, and IFF. Future work includes co-culture with naive and activated T cells to observe immune cell trafficking in the stromal networks as well as integration of the stromal networks into microfluidic devices to mimic the spatial organization of the LN.

Presentation: Poster

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Microgravity-induced mitochondrial dysfunction in human iPSC-based 3D cardiac microphysiological system

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Spaceflight has been shown to have a negative impact on the heart and the cardiovascular system, but the cell and tissue scale effectors of this negative impact remain poorly understood. To address this, we developed a high-throughput microphysiological model of human cardiac tissue, derived from human induced pluripotent stem cells (hiPSCs), to investigate the effects of microgravity on cardiac structure and function. To generate engineered heart tissues (EHTs) that are physiologically-representative of human myocardium, we used a biocompatible and cardiac-specific decellularized extracellular matrix (dECM)-based electroconductive composite scaffold. These dECM hydrogels contain tissue-specific ECM proteins, obtained through decellularization, and when combined with reduced graphene oxide, they recapitulate native tissue-like stiffness and electroconductivity. Our physiologically relevant EHTs were successfully launched to the International Space Station (ISS) where they remained for 28 days while being measured in real time using a magnetic force sensor system before being returned to Earth. Analysis of flight sample data revealed a degradation of contractile function in EHTs in the form of reduced twitch forces and an increase in arrhythmias. Transmission electron microscopy images reveal lipid accumulations and mitochondrial defects in spaceflight samples. This correlates with previous studies showing that mitochondrial dysfunction acts as a central hub for spaceflight-induced damage. A random positioning machine is used to simulate microgravity, and we compare the effects of simulated and actual microgravity. Finally, recent progress on mitigation strategy assessment is discussed.

Presentation: Oral

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Bone marrow microphysiological system to study the sequence dependant haematological toxicity of combined BRD4/BCL-2 inhibition

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Drug combinations as personalized cancer treatments has been a popular strategy in the field of oncology to target the molecular heterogeneity of tumours. However finding tolerable and efficacious oncology drug-combinations in the clinic can be challenging, particularly when compounds have overlapping bone marrow (BM) toxicity. Management of BM safety may be achieved by adjusting combination doses and drug-sequencing/schedules. We have developed a humanized *in vitro* bone marrow microphysiological system (BM MPS) that recapitulates the aspects of living bone marrow for up to 6 weeks. It maintains stem/progenitor cells with simultaneous differentiation into erythroid, myeloid and megakaryocyte lineages and thus can capture the lineage specific haematotoxicity associated with oncology drugs.

AstraZeneca has developed a highly potent BET/BRD4 inhibitor AZD5153 that causes downregulation of critical oncogenes MYC and BCL-2, impacting the epigenetic program. The combination of AZD5153 and Venetoclax (approved BCL2 selective inhibitor) is an exciting opportunity for the treatment of blood cancers. To investigate the BM risk, cells in BM-MPS were treated with AZD5153 and Venetoclax, alone, concurrently, or sequentially (1 week on, 1 week off, or 2 weeks on, 2 weeks off; either AZD5153/venetoclax first). Concurrent treatment induced exacerbated toxicity against erythroid, myeloid and megakaryocyte lineages compared to monotherapies alone ($p < 0.001$), which was mitigated by sequential-dosing ($p < 0.001$). Interestingly, the order of sequential dosing was more important than duration for toxicity mitigation; myeloid toxicity induced by concurrent dosing was significantly reduced ($p < 0.001$) when AZD5153 was dosed first for 1/2 weeks, whereas concurrent dosing-induced megakaryocyte toxicity was significantly reduced ($p < 0.001$) when drug venetoclax was dosed first (either duration). These data indicate that BM-MPS has utility for exploring different dosing regimens to inform on tolerable clinical dosing schedules and paving the way for refinement and replacement of animal studies.

Presentation: Oral

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A microfluidic bone marrow chip for the safety profiling of complex large molecules in preclinical drug development

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Hematologic toxicities are common dose-limiting adverse events during drug development. Highly species-specific therapeutic antibodies have limited the usability of classical animal models for preclinical safety assessment due to insufficient cross-reactivity to non-human homologous proteins. Hence, a human bone marrow microphysiological system (MPS) on the basis of TissUse's HUMIMIC Chip2 technology was developed and assessed for its capability to predict hematopoietic liabilities in preclinical development of complex antibodies such as T-cell bispecifics (TCBs).

A zirconium oxide scaffold mimicking the structure and surface of trabecular bone was seeded with bone marrow mesenchymal stromal cells (BM-MSCs) in a static pre-culture. Human $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) were added into the stromal cell-covered scaffold and subsequently placed into the HUMIMIC Chip2, where the complete model was cultured for up to 31 days. The differentiation and maturation of HSPCs into myeloid, erythroid and lymphoid blood cells could be controlled by the addition of different hematopoietic growth factors. In line with literature reports, adding pro-inflammatory factors and cytokines such as IL1b impacted lineage balance and output on the chip. Furthermore, inhibition of erythroblast differentiation by bispecific TfR-engaging antibodies previously overserved in cynomolgus monkey studies was recapitulated on chip.

For the safety assessment of TCBs, T cells were added to the bone marrow chip. Treatment with TCBs specific for targets on hematopoietic cells were tested in an autologous and an allogeneic setup. In both configurations, application of TCBs resulted in T cell activation and killing of target cells. However, the T cell response level was significantly stronger in the allogeneic setup compared to the autologous setup, indicating that the therapeutic index for TCBs could be underestimated when working with allogeneic cells.

In summary, this study provides proof-of-concept that this MPS for *in vitro* hematopoiesis could be applied for safety profiling of complex large molecules.

Presentation: Oral

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Scaffold-free colonic intestinal spheroids: Preliminary design for IBD disease modelling

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The inflammatory bowel disease (IBD) includes gastrointestinal chronic pathologies (Crohn's disease and Ulcerative colitis) is a prolonged inflammatory state with a progressive impairment of intestinal barrier integrity. Patients' biopsies showed immune cells involvement in epithelial lesions mirroring the complexity of immune-mediated disease.

Intestinal 3D scaffold-free spheroids were produced by the hanging drop technique using a co-culture of primary colonic fibroblasts and primary epithelial colonic cells that spontaneously formed a complex micro-physiological system with a stromal core surrounded by epithelial cells with strong cells-matrix interactions.

The aberrant vicious cycle of gut - immune system crosstalk at the origin of IBD was simulated by a direct exposure of human colonic spheroids and by an indirect multistep approach including THP-1 cells with three different pro-inflammatory stimuli settings. The preliminary results showed the following biological responses:

- the direct exposure of intestinal spheroids to IL-1b (10 ng/mL) for 24 h significantly increased IL-1b release (by ELISA) mirroring an acute pro-inflammatory status.
- inflamed gut mediators showed an impact on immune system: addition of conditioned media from inflamed spheroids to THP-1 cells for 48 h leaded to the up-regulation of ICAM-1 (qRT-PCR) and IL-1b increase suggesting a phenotypical switch of THP-1 monocytes in immunocompetent cells.
- The conditioned media from activated THP-1 was added to healthy intestinal spheroids to mimic a possible indirect pro-inflammatory stimulus on gut tissue mediated by immunocompetent cells: the high IL-1b pro-inflammatory cytokine secretion and gene upregulation with a significant impairment of epithelial barrier integrity was confirmed by dotted and fragmented ZO-1 expression by whole mount IF.

Difficulties in reproducing the IBD vicious cycle in a static system, these preliminary results could be considered as encouraging and a starting point to optimize the experimental design aiming to reproduce in a more relevant and dynamic system the complexity of IBD mechanisms.

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 opioid alternatives for pain management leading federal agencies to fund development of novel approaches to analgesic characterization. We have developed novel, embryonic rat and human pluripotent stem cell-derived, 3D, *in vitro*, microphysiological nerve tissue systems modeling the primary pain signal relay from the peripheral to the central nervous systems through synaptic transmission in the spinal cord dorsal horn (SCDH). In contrast to emerging neural assembloid technologies, peripheral and central neurospheres remain separated by two millimeters, connected solely through peripheral axon extensions, enabling selective stimulation and bioelectric recording of each population in isolation.

We first defined methods to a derive a highly neuronal human SCDH population from pluripotent stem cells (hPSC-SCDH) characterized by low spontaneous activity, multiple excitatory and inhibitory SCDH subpopulations, and pain-related functional markers akin to native SCDH. We then characterized the emergence of circuit-level neurophysiology in our bioengineered 3D microphysiological system when hPSC-SCDH neurospheres were cocultured with commercially-obtained hPSC-derived peripheral nociceptor neurospheres. Similar to our previous findings with embryonic rat SCDH and dorsal root ganglion neurosphere cocultures, peripheral nerve stimulation generates synaptically-evoked bioelectric field potentials in cocultured SCDH neurospheres that are differentially modulated by morphine and other common, mechanistically-distinct analgesics.

Synaptic field potentials are also observed when recorded through integrated, polycarbonate/stainless-steel, 3D microelectrode arrays (MEAs) that use novel silicon dioxide-based functionalization, though full electrode characterization and optimization of MEA integration into the culture system remain in progress. MEA integration will enable repeated or continuous data collection from multiple recording sites within our nerve constructs over time, significantly multiplying quantity and complexity of the physiological information generated. 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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Scaffold-free cartilage spheroids model: Application to investigate chondrocytes regenerative capacity

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Rheumatoid arthritis and osteoarthritis are the most common cartilage diseases, with causes not fully cleared. Due to an aberrant pro-inflammatory stimulus, the immune system attacks the synovial membrane of the joint capsule, leading to inflammation and swelling until joints cartilage and bone destruction.

The main therapeutic approaches are based on allogenic chondrocytes transplantation for tissue regeneration and synovial membrane restoration, but several limitations were observed in chondrocytes stabilization *in vitro*. Once obtained, committed pre-chondrocytes need to be re-differentiate in mature cartilage cells to allow tissue regeneration. Due to difficulties in cells establishment and the employment of an exogenous matrix to force tissue maturation, more alternatives are sought.

Primary human chondrocytes at early passage were cultured by hanging drop technique to produce a miniaturized cartilage micro-physiological system based on scaffold free spheroids mimicking native human cartilage thanks to the establishment of a physiological niche. The tridimensional configuration provides the geometrical guidance to preserve re-differentiative capability leading to a mature cartilage tissue close to the natural counterpart.

After 7 days of culture, chondrocytes spheroids reached a full differentiation, expressing key biomarkers of natural cartilage profile. The high metabolic activity by ATP quantification showed a preserved viability and an active cellular signaling in long-term culture, unlike conventional spheroids models based on exogenous micro-scaffolds. The regenerative capability was confirmed by expression of key mediators in whole mount IF.

The expression of collagen type II suggested a complete chondrocytes differentiation and a stromal development, thanks to their micro-physiological niche. In addition to this, fully re-differentiated spheroids preserved their re-generative potentiality, confirmed by the expression of S100, a critical molecule of cartilage activation.

Our results highlight that the tridimensional endogenous micro-environment for cartilage modeling provides more physiological instructions to preserve natural cartilage regenerative properties laying the groundwork for disease modeling and personalized therapies.

Presentation: Poster

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Development of a human iPSC-derived sensory neurons and human primary keratinocytes *in vitro* model in a microfluidic device allowing physiological cell interactions

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Psoriasis, atopic dermatitis, and skin sensitivity syndrome are pathologies with increasing prevalence and that involve both skin cells, i.e., keratinocytes, and sensory neurons. However, there is still not many effective treatments, partly because of the lack of relevant *in vitro* model. Co-culture models often use rodent neurons cells, and do not fully recapitulate the anatomical structure, i.e., neuronal cell body compartmentalized from the innervated skin. Here, thanks to a compartmentalized microfluidic device, we developed an human cell model reproducing i/ skin compartment with nerve endings and keratinocytes microenvironment and ii/ spinal cord compartment with neuronal cell body.

The device consisted of two isolated channels linked by multiplexed 450 µm long-microchannels in which only axons can pass through over dendrites. Such configuration allows the creation of a selective barrier, whilst connected, between the cell cultures.

Human iPSC-derived sensory neurons were seeded in one channel, and human primary keratinocytes were seeded in the other. Cell seeding density and timing between the two cell types were optimized to ensure proper cell maturation and proliferation. Thanks to compartmentalization and fluidic isolation, cells were cultivated with their respective culture medium allowing optimal growth. After 28 days *in vitro*, cells were independently characterized by immunofluorescence of TRPV1, CGRP, K10, K14 and Synapsin.

The developed compartmentalized human *in vitro* model confirmed the expression of the skin tissue specific markers, enabling access to nociception, itch or inflammation modulation for versatile skin disease models. It could therefore be used to test activity of molecules on either soma or axonal projections and better understand biological mechanisms of action of a preclinical stage compound. Furthermore, the microfluidic device can be coupled to microelectrode array (MEA) technology allowing electrical signals recoding of soma after axonal molecular stimulation, leading to innovative readout for the dermo-cosmetic active research.

Presentation: Poster

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Human brain-organoids-on-chip: Advanced microfluidic device for reproducible organoids culture

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There is an urgent need for translational preclinical models to enhance successful clinical trials outcomes, particularly for neurological troubles innovative therapeutics. Alternative methods, such as predictive human based *in vitro* models are particularly in the spotlight of Regulatory Bodies. Organs- and Organoids-on-Chips (OoCs) technology have shown the great potential to reduce the cost and ethical burden of animal studies. Cerebral organoids now appear as great potential for understanding complex biological processes with the ability to mimic and recapitulate many features of the human brain.

One of the main current challenge is the cell apoptosis described in the center of cerebral organoids leading to a lack of maturation. This is caused by a lack of oxygenation and nutrient supply in the center of this 3D structure. Our objective is to propose an innovative microfluidic system allowing the generation of reproducible cerebral organoids thanks to (i) a cell culture protocol entirely realized in microfluidics, (ii) the contribution of a controlled flow and (iii) the compatibility with automation processes in order to meet the expectations of scientists and pharmaceutical companies.

Here, a technology to perform 3D cell culture in a 3D-Deposition microfluidic chamber coupled with smart membrane perfusion has been presented. Characterization of the fluid flow in the thick three-dimensional cell culture perfusion was shown. Standard Operating Procedure for the culture and maintenance of cerebral organoids in microfluidic devices has also been established. Our data highlight the compatibility of the device with pharmacological medium-throughput readouts.

In conclusion, 3D microfluidic *in vitro* models open new field applications such as assessment of drug distribution as alternative to animal testing. Thus, a design of interconnects with and without permeability membrane of 3D-deposition microfluidic chambers as multi-Organoids-on-chip will allow to study ADME and the delivery and screening of molecules that target brain under more physiologically relevant conditions.

Presentation: Poster

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A continuous, automated perfusion culture and analysis system (CAPCAS) to enable massive parallelization of organs-on-chips, chemostats, and other miniature bioreactors

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Application of microphysiological systems (MPS) models to pharmacology, toxicology, and systems biology is limited by logistical and economic difficulties associated with increasing how many MPS assays can be conducted simultaneously. In contrast, a single pharmaceutical facility for automated high-throughput screening of drugs can use a short-duration, well-plate-based, seed-expose-measure sequence to study ~80,000 different drug-cell interactions daily. One appeal of single- and coupled-organ MPS assays is their recapitulation of human physiology *in vitro*, with high-content imaging and multi-omic characterization of perfused mixed-cell populations cultured for long periods of time in microenvironments that are much more physiologically relevant than 2D monocultures on plastic. While a few MPS technologies support 96 assays in the footprint of a standard well plate, most commercial systems use pumps, rockers, or pressurized reservoirs to maintain a small number of individual or coupled organ chips. The extended duration of iPSC differentiation and maturation of both iPSCs and complex tissue architectures presents new challenges in automated, long-term fluidic control that currently limit MPS throughput. With our microfluidic pumps and valves, we have created Multi-Well MicroFormulators that superimpose different, realistic pharmacokinetic profiles on each well of a 24- or 96-well plate, now in commercial use as the CN Bio Innovations PhysioMimix system. We report the development of fourth-generation pump and valve cartridges that are compact, easy to assemble, use, and refurbish, and that have allowed us to create a first-in-class, continuous, automated perfusion culture and analysis system (CAPCAS). Our 12-channel chemostat system will soon be extended to support 48 chemostats on a single plate and ultimately over 1,000 chemostats in a nine-deck incubated instrument rack, all under control of artificial intelligence, machine-learning software, to create a robot-scientist that functions as a self-driving biological laboratory. Our technologies will readily support long-term, massively parallel MPS studies.

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Presentation: Oral

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3D Nephroscreen: High throughput drug-induced nephrotoxicity screening on a microfluidic proximal tubule model

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Renal toxicity remains a major issue in clinical trials, and stresses the need for more predictive models fit for implementation in early drug development1. Here, we describe the use of a high throughput, microfluidic platform for the detection of drug-induced nephrotoxicity. A microfluidic platform (Mimetas' OrganoPlate®)2 was combined with renal proximal tubule epithelial cell lines (PTEC) and exposed to fluid shear stress. A 12-compound nephrotoxicity screen across multiple laboratories was performed in collaboration with sponsors and the NC3Rs3.

ciPTEC-OAT1 or RPTECs (Sigma) seeded against an ECM gel under perfusion flow were used to establish a proximal tubule-on-a-chip. Tubules with polarized epithelium containing functional transporter expression were obtained. Drug-induced toxicity was assessed by exposing the tubules to 4 benchmark compounds with known clinical effect and 8 blinded compounds supplied by the sponsors for 24 and 48 h. Epithelial barrier tightness and drug-transporter interactions were evaluated. Parallel to this, cellular damage and stress were assessed using various read-outs. Finally, gene expression analysis was performed to assess AKI markers. The Nephroscreen revealed that a combination of cell viability, LDH and miRNA release were the most predictive readouts in determining nephrotoxicity. Most of the blinded compounds resulted in toxicity detected by at least one of the functional read-outs.

Nephroscreen provides a reliable standardized and automatable system for efficacious identifying nephrotoxicants and revealing their mode of action.

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Presentation: Oral

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Application of single cell transcriptomics of MPS for pre-clinical safety assessment

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Enhancing the early detection of safety liabilities of new therapies would advance drug development. Human Microphysiological systems (MPS) provide an opportunity to improve preclinical-to-clinical translation and emerging data from these models demonstrate the potential of MPS to transform safety assessment and enhance the human-relevance of our pre-clinical tests.

Finding tolerable and efficacious oncology drug-combinations in the clinic can be challenging with bone marrow (BM) toxicity often limiting. Managing BM toxicity may be achieved by adjusting combination-doses and drug-sequencing/schedules, but it can be time-consuming finding the best regimen for patient-benefit. To guide this we have developed a human BM MPS that maintains stem cells with concurrent differentiation into erythroid, myeloid and megakaryocyte cells. We conducted single cell RNA sequencing (scRNAseq) to provide precise characterization of cells in the BM MPS across three hematopoietic stem cell (HSC) donors and over time (0, 3, 7 and 14 days).

The results showed transcriptional similarity of MPS cells to human BM cells across multiple donors and timepoints. All expected cell types were present and cell abundance reflected differentiation state (e.g., early erythroblasts were more abundant at earlier timepoints, while erythroblasts were more abundant later), supported by pseudo-time analysis.

Interestingly, differences between HSCs were identified, indicating a transcriptional bias towards either the granulocyte or megakaryocyte/erythroid lineage, and drug-target gene expression associated with lineage-specific BM toxicity also differed between lineages.

The transcriptional similarity to *in vivo* human BM cells supports the use of the MPS as a physiologically-relevant model, and our analysis provides a framework for generating hypotheses about lineage-specific drug toxicity, and more generally illustrates the power of large datasets to derive novel testable biological hypotheses. Ultimately, scRNAseq analysis may provide an alternative approach for the use of MPS in decision-making for drug projects.

Presentation: Oral

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Evaluating drug-induced liver toxicity of acetaminophen, trovafloxacin and levofloxacin in a triple-cell microphysiological liver sinusoidal model

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Drug-induced liver injury (DILI) comprises hepatic adverse events with diverse clinicopathologies and frequent drug withdrawal. Therefore, we developed a microphysiological model of the human liver sinusoid to provide a suitable *in vitro* platform to evaluate DILI. This study investigated the toxicity of trovafloxacin (TVX), the non-DILI drug levofloxacin (LVX) and acetaminophen (APAP) at human-relevant doses.

The model comprised two cultivation chambers in the Dynamic42 biochip. The vascular (top) chamber incorporated upcyte® liver sinusoidal endothelial cells and monocyte-derived macrophages. The hepatic (bottom) chamber consisted of HepaRG™ hepatocytes. The model was cultured under vascular perfusion with daily treatment for up to 7 days. Immunofluorescence staining for vascular and hepatic markers was performed. Live cell staining for glutathione and reactive oxygen species (ROS) were used to survey specific toxicity mechanisms. Medium effluents were analyzed for lactate dehydrogenase (LDH), alanine transaminase (ALT) and cytokines.

Stimulation of the model with TVX and APAP resulted in dose-dependent loss of vascular and hepatic tissue integrity, reduction of marker expression and decline in cell viability. Furthermore, increased LDH and ALT were measured after treatment with TVX and APAP. Augmented glutathione depletion and accumulation of mitochondrial ROS were in addition detected after live cell staining. Trovafloxacin induced an increase in proinflammatory cytokines in the vascular chamber, demonstrating an immunomodulatory effect. In contrast, LVX treatment did not induce toxicity within the model.

In summary, the occurrence of drug-induced liver toxicity was suggested for APAP doses $\geq$ 1 mM and TVX doses $\geq$ 10 µM. Treatment with the drug LVX did not indicate hepatotoxicity up to 20 µM in our model. This work highlights the capability of our *in vitro* model to detect DILI and to disclose the underlying toxicity mechanisms. Concluding, these results highlight the urgency to advance microphysiological models for improved and cost-efficient drug safety testing and reduction of animal harm.

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Towards a comprehensive osteoarthritis modelling on-chip: Controlled mechanical stimulation in bi-layered micro-tissues compartments

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Osteoarthritis (OA) is a multifactorial joint disorder and a major cause of disability worldwide, with an increasing burden on the ageing population. Despite a deeper comprehension of OA acquired in recent years, currently available treatments only rely on symptoms attenuation. Relevant preclinical disease models are thus needed to assist the development of disease modifying OA drugs (DMOAD).

Various *in vivo* models of OA have been proposed. However, differences in animals and human biology, joint biomechanics, and OA onsets modalities have so far limited effective translations of new compounds to the clinical setting. Furthermore, the ease of manipulation and the possibility of avoiding confounding factors render OA study through human *in vitro* modelling particularly desirable. We recently developed a human cartilage-on-chip (CoC) model able to recapitulate important OA clinical traits through the sole application of a mechanical hyperphysiological compression, demonstrating its suitability in predicting the anti-inflammatory effect of known compounds in agreement with clinical data (Occhetta et al., 2019).

Despite cartilage breakdown represents the most prominent feature in OA, the whole osteochondral unit (OCU) – constituted by cartilage and subchondral bone – is affected, although the primary causing factors (e.g., mechanical overloading or inflammation) and the sequence in which OA modifications occur in the different tissues remain unclear.

Here we introduce a newly designed Organ-on-Chip (OoC) concept, namely the vertical burst valve (VBV), to expose complex biphasic microtissues to discrete compressive strains akin to those experienced by OCU tissues *in vivo*. Such technology enabled to achieve different mechanotransduction and inflammatory/degradative responses in human cartilaginous microconstructs. Finally, we provided a proof of concept of how local mechanical alterations in the subchondral layer affect cartilage response to loading. The proposed OoC model may speed up the screening of DMOAD candidates providing a human clinically relevant setting.

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Alveolar and bronchial human microphysiological systems for use in respiratory infection research and therapeutics evaluation

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There is an immediate need for better preclinical lung models. Respiratory diseases make up 3 of 10 leading causes of loss of life, and yet only 3% of new pulmonary therapeutics reach the market to alleviate these diseases. The advent of the COVID-19 pandemic has further realized the necessity of more physiologically relevant models which can allow rapid identification of treatments.

Two novel primary human cell lung models have been developed for this purpose using a microphysiological system in an open well Transwell format, air-liquid-interface, and perfused media. The physiological conditions cause the alveoli model to create alveolar sac structures containing AT1 and AT2 cells, and the bronchial model to form a stratified tissue that produces mucus and cilia. Compared to traditional static cultures the perfused models produce superior cellular phenotypes and tissue structures. Differentiation into key cell types were increased, both in alveolar tissues (AT1 (3-fold), AT2 (7-fold)) and bronchial (Club (21-fold) and Goblet cells (7-fold)).

Further complexity was added to the lung models by including primary human endothelial cells on the basolateral side of the perfused Transwell. Monocytes were also incorporated either basolaterally in the bronchial model to represent circulating immune cells or on the apical side to mimic alveolar macrophages in the alveolar model.

Pseudotyped lentivirus expressing the SARS-CoV-2 Spike protein was used to infect the models to validate their use in COVID-19 research. Infection and subsequent elevated IL-6, TNFα and IL-1β responses were mapped during the infection, demonstrating the relevance of the model compared to patient inflammatory profiles in COVID-19 disease. Infection was inhibited using anti-SARS-CoV-2 Spike RBD neutralizing monoclonal antibodies, where the model responded in a dose-dependent manner. These data demonstrate the ability of the model to predict the efficacy of therapeutics against SARS-CoV-2, as well as facilitate further understanding of COVID-19 disease progression.

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Lymphangion-chip experimental platform supports co-culture and signaling between lymphatic endothelial and mural cells

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The pathophysiology of several lymphatic diseases, such as lymphedema, depends on the function of lymphangions that drive lymph flow. Even though the signaling between two main cellular components of a lymphangion, Lymphatic Endothelial Cells (LECs) and Lymphatic Muscle Cells (LMCs), is responsible for crucial lymphatic functions, there are no *in vitro* models that have included both cell types in a physiologically relevant environment. Here, a fabrication technique (Gravitational Lumen Patterning or GLP) is developed to create Lymphangion-Chip. This organ-on-chip consists of co-culture of a monolayer of endothelial lumen surrounded by multiple and uniformly thick layers of muscle cells. The platform allows construction of a wide range of luminal diameters and muscular layer thicknesses, thus providing a toolbox to create variable anatomy. In this device, lymphatic muscle cells align circumferentially while endothelial cells aligned axially under flow, as only observed *in vivo* in the past. This model further demonstrated a robust sensitivity of this relative alignment of the two cells with respect to the presence or absence of co-culture and mechanical forces (shear stress), thus suggesting that LECs and LMCs are biologically and functionally active within the chip. This system successfully characterizes the dynamics of cell size, density, growth, alignment, and intercellular gap due to co-culture and shear. The time-dependent decrease in the subendothelial gap strongly suggests proactive LEC-LMC signaling as LECs and LMCs grow and proliferate within the device. Finally, exposure to pro-inflammatory cytokines reveals that the device could produce the regulation of endothelial barrier function through the lymphatic muscle cells. Therefore, this organ-chip technology allows researchers to include essential lymphatic vascular components in a tunable 3D microphysiological system. This bioengineered platform is suitable for use in preclinical research of lymphatic and blood mechanobiology, inflammation, and translational outcomes.

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Development and evaluation of immune competent dynamic lung-on-chip model for the prediction of lung inflammatory toxicity

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Inhalation is an important delivery route for therapeutic agents to treat respiratory diseases such as asthma and chronic obstructive pulmonary disease. The utility of the inhaled route for drug delivery presents a great advantage in achieving direct access to the lung but is frequently impacted by clinical dose level limitations due to lung pathology or functional respiratory effects in non-clinical safety studies. In order to ensure that candidate drugs have the right safety profile, we aim to predict and mitigate lung safety concerns as early as possible during the drug delivery process. A key gap in the arsenal of *in vitro* methods that can be applied routinely is to predict adverse inflammatory responses in the lung induced by inhaled modalities. Therefore, sensitive and predictive lung models including immune components are required as these represent significant drivers in the lung inflammatory response. In collaboration with the research team of AlveoliX AG, we have evaluated the human AXLung-on-chip model as an *in vitro* tool to predict lung inflammation and toxicity. The chip set up enables application of physiological stretch mimicking human breathing and incorporates cell types such as epithelial, endothelial and immune cells. We investigated different treatment regimens using a set of compounds with known *in vivo* lung toxicity profiles. The main endpoints utilized for evaluation were secreted cytokines, cytotoxicity and Trans Epithelial Electrical Resistance (TEER). Through the incorporation of multiple endpoints and a comparative analysis of static vs dynamic conditions, we were able to reproduce *in vivo* inflammatory histopathological findings and distinguish between compounds that cause this *in vivo* and compounds that do not. This pilot study shows potential for the usage of the AlveoliX Lung-on-chip model for predicting *in vivo* lung inflammatory toxicity and warrants further studies to understand the quantitative translation from this *in vitro* model to clinical safety.

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A novel high-throughput assay for cell migration, chemotaxis and function

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Response to a chemotactic gradient within the extracellular matrix (ECM) governs the activity of numerous immune cells manifesting as their migration and function into and out of tissue microenvironments. Consequently, modulating the chemotaxis machinery could draw in or exclude desired immune cell type(s) from diseased tissue microenvironments resulting in useful therapeutic outcomes.

While the majority of chemotaxis within the human body occurs within three-dimensional, multi-cell rich extracellular matrix spaces, conventional assays utilize transwell based platforms that largely ignore it. Furthermore, the functional activity of the responding cells cannot be thoroughly investigated within these experimental set-ups. There is a lack of high throughput screening platforms that allow studying and modulation of 3D cell migration and function within native ECM environments and their interactions with different tissue resident cell types.

Existing microfluidic models that study chemotaxis and 3D cell migration either lack the throughput essential for industrial drug discovery and development or are increasingly laborious to create and work with. Herein, we present a novel platform technology and a screening amenable assay design that captures 3D cell migration through native tissue-like ECM environments within high-throughput microfluidic well plates (96 individual experiments within a plate) with a confocal imaging-based readout.

Activated $CD3^+$ T-cells stained with far red nuclear stain responded to the chemokine gradient generated within the ECM by migrating into the microfluidic channel which were detected using confocal microscopy. Further, the activated $CD3^+$ T-cells showed a concentration dependent chemotaxis into the ECM filled microfluidic chambers across a wide array of chemotactic stimuli such as recombinant chemokines (CXCL12) and small molecule chemokine receptor agonists. Overall, this assay platform represents a significant leap of innovation towards generating physiologically relevant high-throughput *in-vitro* assays that allow studying and modulation of mammalian cell migration and function within multicellular complex tissue microenvironments to achieve therapeutic outcomes.

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The development of a high-throughput screening platform to identify ovarian endocrine disrupting chemicals using a 3D alginate encapsulated *in vitro* follicle growth system

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The ovary is the female gonad and contains various stages of follicles as the functional unit. Once activated, follicles develop from the preantral to antral stage to reach maturation, secrete hormones, and ovulate oocytes to maintain female reproductive cycle and fertility. Both environmental chemicals and clinical drugs can cause ovarian toxicity and heighten women's risks of infertility and ovarian diseases, such as premature ovarian failure, polycystic ovarian syndrome (PCOS) and ovarian cancer. However, identification of chemicals that are ovarian toxic is challenging due to the low throughput of animal models and the use of ovarian cell lines that do not faithfully represent ovarian functions. We established a 3D alginate hydrogel encapsulated *in vitro* follicle growth (eIVFG) model which can both phenotypically and mechanistically recapitulate key ovarian events as occurring *in vivo*, including follicle maturation, hormone secretion, and ovulation. Here, we aimed to use the eIVFG model to develop a high-throughput screening platform to identify ovarian endocrine disrupting chemicals (EDCs). We first developed a vitrification system to cryopreserve mouse follicles and demonstrated that vitrified follicles grown in eIVFG had reproductive outcomes and follicular transcriptomic profiles comparable to fresh follicles, enabling a high-content follicle biobank. We next used the eIVFG model to test 42 chemicals, including 10 bisphenol analogues, 7 flame retardants, 6 per-and polyfluoroalkyl substances (PFASs), 6 harmful algal bloom (HAB) toxins, and 8 pre-clinical compounds. Results showed that several tested chemicals exhibited ovarian disrupting effects. For example, long-chain PFASs inhibited follicle ovulation by disrupting proteolytic signaling pathways; HAB toxin microcystin-LR suppressed follicle maturation to cause defective ovulation; and AZD7762, a checkpoint kinase inhibitor, promoted follicle death by inducing granulosa cell apoptosis. Taken together, our study demonstrates that eIVFG is a powerful tool to develop a high-throughput screening platform to identify ovarian EDCs and also investigate the toxic mechanisms involved.

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Development of a perfusable vascularized micro organ system for the study of vascular biology and the vascular niche

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Current preclinical studies rely heavily on 2D *in vitro* models or murine *in vivo* models that fail to recapitulate human disease biology and the behavior of drugs in the human body. To address the prevailing dissonance between preclinical models and human studies, we have developed the Vascularized Micro Organ (VMO) – a unique *in vitro* microphysiological system that supports the *de novo* formation of a 3D capillary bed in an extracellular matrix. The microvasculature forms over 4-5 days, anastomosing with endothelial cells (ECs) lining the arteriole and venule that flank the tissue chamber to form a coherent perfusable network. These nascent vessels are free to sprout, regress and otherwise respond to changes in environmental cues like shear stress. The VMO allows for physiologic, microvessel-mediated delivery of nutrients and drugs to the surrounding stroma. Moreover, the human-derived ECs can be easily manipulated – by siRNA, shRNA, lentiviral transduction, etc. – to model vascular pathologies. Based on this VMO platform, we have developed models to evaluate vascular diseases such as atherosclerosis, Port Wine Birthmark, and COVID-19 infection. Additionally, the VMO enables the study of tissue-specific environments like the blood-brain barrier and vessel-tumor interactions. Only more recently has the vasculature been appreciated for its transcriptomic and functional diversity, including the unique microenvironment it generates and its contribution to organ development, homeostasis, and regeneration. Here we demonstrate the ability of the VMO and its derivative models to capture these complex vascular functions, which can be applied not only to interrogate disease biology, but also evaluate novel therapeutics.

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Using *in vitro* cell models, kidney MPS, and PBPK modeling to predict human renal clearance: Health, disease, and drug interaction

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Modeling human renal clearance is difficult in healthy populations; predicting altered clearance in subjects in the setting of renal impairment and/or drug interaction is even more complicated and can be rate-limiting for drug development and regulatory approval. In order to facilitate these processes, we have used human renal transporter engineered cell lines to quantitate *in vitro* pharmacokinetic transport and inhibition parameters for drug substrates and interacting compounds. A dual-channel "vascularized" human proximal tubule MPS can then be used to estimate renal clearance by determining vectorial transport rates (Chapron et al., 2020; Imaoka et al., 2021). The combined data can be scaled and used to populate a multi-compartment physiologically based pharmacokinetic (PBPK) model (Huang and Isoherranen, 2020) that incorporates kidney disease parameters and/or inhibitory compounds (drugs, uremic solutes).

For proof of concept, we used this methodology to model the renal clearance of p-aminohippurate in healthy subjects with an average fold error of 1.96. In an application with clinical relevance, we accurately modeled the renal clearance of morphine and morphine 6-glucuronide (Imaoka et al., 2021) with an average fold error of 1.22 to 1.30 in chronic kidney disease patient simulations (both within a pre-defined 2-fold model acceptance criteria). We are currently using this method to model uremic solute-mediated inhibition of tenofovir renal clearance, which represents both disease and drug-inhibition scenarios.

We believe that MPS technology can be used to bridge the gap between *in vitro* cell models and *in silico* model systems to accelerate the drug development process and provide predictive guidance for medication use in patients with kidney disease and/or complex medication requirements.

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Presentation: Oral

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Patient-specific human immunocompetent adipose tissue-on-chip models for obesity and endocrinology research

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Obesity and other adipose tissue-associated diseases, such as diabetes, have reached epidemic proportions globally. In this era of 'diabesity', adipose tissue has become a target of high interest for therapeutic strategies. Yet, research on human adipose biology is hampered by a lack of predictive model systems. Even though many valuable insights could be gained from animal models, they often fall short of predicting human physiology. Then again, unusual characteristics of mature adipocytes, such as buoyancy, fragility, and large size, make conventional cell culture approaches challenging.

Leveraging Organ-on-Chip technology, we developed microphysiological *in vitro* models of human white adipose tissue (WAT) and beige adipose tissue (bAT): In specifically tailored microfluidic platforms featuring vasculature-like perfusion, we integrated either (i) 3D-tissues comprising all WAT-associated cellular components (mature adipocytes, organotypic endothelial barriers, stromovascular cells including adipose tissue macrophages) in an autologous manner or (ii) human stem cells for on-chip beige adipogenesis. The developed platforms enable recapitulation of pivotal adipose functions, such as energy storage and mobilization as well as endocrine and immunomodulatory activities. Along the way towards highly complex, immunocompetent models integrating almost all adipose-associated cell types, a mix-and-match strategy was established allowing for a flexible combination of cellular modules to fit-for-purpose models tailored to a specific scientific question. Moreover, we compiled a toolbox of readout methods that enables a comprehensive characterization of adipose tissue structure and function on chip, demonstrating tissue functionality for culture times beyond one month. Case studies on compound screening and immune responses highlighted the models' suitability as tools for target identification in drug discovery or for studies on immunometabolism. All in all, the developed models hold great potential for mechanistic studies on adipose tissue biology or disease modelling in the context of obesity and diabetes, as well as for personalized or precision medicine due to their fully autologous character.

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New human Alzheimer's neurons on-a-chip platform enables Big Data generation through a top-down screening approach

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The most common form of dementia is Alzheimer's, which contributes to 60-70% of the 55 million dementia cases (WHO, Sep 2021). Up to 99% of Alzheimer's therapies that showed promising results in preclinical studies failed in clinical trials (Cummings et al., Alzheimer's Research and Therapy, 2014). New and more predicative models are needed to accelerate compound developments in this disease area. At Ananda Devices, we have developed technology to rapidly grow human neurons on a chip, precisely organizing neuronal networks for rapid and robust evaluation of changes in neuronal morphology, biochemistry and network connectivity (Magdesian et al., 2017). The NeuroHTS™ microplates can rapidly evaluate 7+ parameters on neuronal morphology, neurite growth, synapse formation and network dynamics with over 85% plate to plate reproducibility. Here we have developed a human microphysiological system (MPS) to model Alzheimer's neurons on-a-chip. We characterized this Alzheimer's neurons on-a-chip model using a top-down data generation approach and compared it with healthy neurons on-a-chip models. Using standard imaging technology, we quantified 9 phenotypical and biochemical endpoints such as neurite outgrowth, bundle thickness, neurite curvature, branching numbers, etc. for over 3000 neurons. Using automated analysis, we were able to analyze the large quantity of data generated from each neuron. Through this high-definition analysis, we identified 3 key endpoints that distinctively characterized the disease state. The new human Alzheimer's neurons on-a-chip can be deployed at scale for preclinical compound screening or post clinical translational research in identifying key patient populations. With the high level of standardization, reproducibility, and compatibility with automation, it is poised to potentially reveal key individual differences in the heterogenous Alzheimer's patient population. The large dataset generation and automated analysis could aid in generating predicative insights to de-risk future compound development.

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A complex immunocompetent pancreatic islet cell model for TCB safety assessment

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In the context of preclinical development of T cell bispecific antibodies (TCBs), on-target off-tumor as well as off-target off-tumor reactivity to pancreatic cells poses the risk to trigger an acute immune reaction against pancreatic cells, which may result in clinically relevant adverse events such as rapid onset diabetes or acute pancreatitis. To address potential liabilities of TCBs in the pancreatic islets of Langerhans, a co-culture of 3D InSight™ Islet microtissues with allogeneic PBMCs was established. These microtissues are generated from primary human pancreatic islets cells. Several technical limitations interfered with a straight-forward approach to set up the co-culture system and readouts. Importantly, classical toxicity readouts such as LDH were found to be non-conclusive and non-relevant and had to be replaced with complex imaging and flow cytometry approaches. Initial attempts to set up the co-culture with allogeneic, HLA-mismatched PBMCs resulted in a significant level of tissue damage in the absence of treatment, and failure of positive and negative controls. This was attributed to a high sensitivity of islet microtissues to alloreactivity. In the absence of the possibility to source autologous material, HLA-A, -B, and -C-matched allogeneic PBMCs were tested. Indeed, matching of MHC-I complexes enabled a co-culture of islet microtissues with PBMCs without impacting microtissue or PBMC viability. The co-cultures were treated with positive and negative control T cell bispecific antibodies (ranging from 0.05 to 50 nM) for 24-72 hours. Reduction of viable target cell counts upon treatment with two positive control antibodies correlated well with increased levels of pro-inflammatory cytokines and Granzyme B in the cell culture supernatant as well as with up-regulation of T cell activation markers. The future development will focus on the inclusion of an islet-specific functional read out as well as on the establishment of an immunocompetent culture system for the exocrine pancreas.

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White and beige/brown human adipose tissue-on-chips as a platform for disease modeling and drug discovery

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Obesity has become a major public health crisis in the United States, with an estimated economic burden of $100 billion annually. The economic and health burden is easily tripled when factoring the increased risks of developing associated co-morbidities such as type II diabetes, cardiovascular disease, cancer and complications as result of infectious diseases (as we observed recently with Covid-19). Once associated primarily with high-income countries, obesity is now prevalent in low- and middle-income countries.

There is a need to develop platforms that provide earlier indicators of toxicity and drug efficacy using human-based systems to accelerate drug development and reduce the need for drug testing in animal models, which is time consuming, costly and often does not predict the adverse effects in humans.

We have developed a proprietary human scaffold – Obagel™ – that, when combined with primary human stromal vascular fraction, creates 3-D tissue engineered white or beige/brown adipose depots called "Fat-on-a-Chip," for disease modeling and drug discovery.

The molecular, functional/physiological, and cellular characterization of our "fat-on-a-chip" system demonstrates a scalable ability to manufacture these "WAT-on-a-chip" or "BAT-on-a-Chip" constructs for modeling white and brown adipose, respectively. Furthermore, we licensed the use of a matrix mimicry system for *in vitro* adipocyte hypertrophy, using fiber networks. These self-assembling adipose depots, maintained for extended culture periods with minimal effort, can be used to model human adipose tissue that is representative of individual donor demographics, including body mass index, age, gender, ethnicity and metabolic disease status.

The use of "fat-on-a-chip" products can have a significant impact on the translation and discovery-based endpoint phases of therapeutic development, thereby minimizing the use of small animal studies required for predicting toxicity and therapeutic responses.

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Modeling doxorubicin's pharmacokinetics and pharmacodynamics in a human InterOrgan chip

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Organs-on-chips, microphysiological platforms with bioengineered human tissues, can mimic organ-level and systemic physiology by replicating key physiological and biological properties. These platforms offer a new paradigm for testing drugs which may lead to a faster and more affordable development of new therapeutic agents and the identification of early biomarkers of human pathophysiology. Studies of pharmacokinetics (PK) and pharmacodynamics (PD) are already being performed in these platforms but establishing them as reliable PK and PD models remains a major challenge. Here we show the use of our organs-on-chips platform for the *in vitro* modeling of doxorubicin's PK and PD (Ronaldson-Bouchard et al., in press). For *in silico* simulations of doxorubicin's distribution and metabolism we developed a computational model of the bioengineered multi-tissue platform. The computational PK model of doxorubicin compared closely with the experimental data, predicting doxorubicin metabolism by the liver tissue, and its diffusion through the platform over time. We also observed changes in the functionality of heart, liver, bone, skin, and endothelial barrier matching the previously reported data, and we identified early miRNA biomarkers of doxorubicin cardiotoxicity, as observed in cancer patients. The PK and PD profiles in the integrated multi-tissue chip demonstrated how microphysiological systems could be used in preclinical PK and PD studies. We anticipate organs-on-chips in combination with *in silico* models may improve the development of new therapeutic agents and facilitate clinical translation of preclinical results. Furthermore, increasingly complex physiological models could be used for studying patient-specific responses to drug toxicity and evaluating new treatments.

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Presentation: Oral

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Identifying RNA biomarkers of radiation injury with a liver-on-a-chip model

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Radiation exposure can damage the liver, leading to acute and chronic conditions such as radiation-induced liver disease and fibrosis. These conditions can impact quality of life and may lead to death, necessitating early interventions determined by injury biomarkers from accidental exposure. The goals of this study are twofold: (1) establish a microfluidic liver-on-a-chip device as a physiologically relevant model for studying radiation-induced tissue damage; and (2) determine acute changes in RNA expression and biological pathway regulation to elucidate biomarkers of radiation injury. To model functional human liver tissue, we established a co-culture of human liver sinusoidal endothelial cells (LSECS) and hepatocytes using Emulate's liver-on-a-chip system. The chips were subject to 0 (sham), 1, 4, or 10 Gy irradiation. Cells were collected at 6 h, 24 h, or 7 days post-radiation for RNA isolation. To identify significant RNA biomarkers, we performed whole transcriptome analysis via RNA-Seq. Changes in time, dose, and cell-type yielded distinct expression patterns. Pathway analysis indicated significant deregulation of cell cycle, DNA replication, and repair pathways, demonstrating the utility of this culture system to model typical radiation responses. Overall, hepatocytes showed little response to radiation, with fewer differentially expressed genes than in endothelial cells. We found commonly expressed genes between the LSECs and our past *in vitro* experiment with human coronary artery endothelial cells, such as HtrA serine peptidase 1(HTRA1), sulfatase 2 (SULF2), pleckstrin homology like domain family A member 3 (PHLDA3) and growth differentiation factor 15 (GDF15). Also, we show that 6h after radiation may be too early to identify radiation damage via RNA biomarkers; however, consistent differential expression occurred at 24 h and 7 days. This study demonstrates that organ chips may provide valuable information for studying organ-specific radiation injury, with greater flexibility and physiological relevance than two-dimensional *in vitro* and animal models.

Presentation: Poster

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Peristalsis-like deformations increase tumor cell intravasation through GABAergic signaling in a colorectal cancer-on-chip model

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Colorectal cancer (CRC) is one of the deadliest cancers in the U.S., yet we understand little about the mechanisms behind this disease. We are developing an organ-on-a-chip (OOC) model that recapitulates the complex nature of cancer progression to increase our understanding of CRC and accelerate the discovery of new treatments. Emulate, Inc.'s OOC technology maintains physiologically relevant aspects of organ structure and function by incorporating tissue compartments and mechanical forces to mimic *in vivo* peristalsis and fluid flow. We have adapted this platform to create a CRC-Chip that consists of endothelial cells in the bottom channel and normal colon epithelial cells plus fluorescently-labeled CRC cells in the top channel. Via confocal microscopy, we observe cancer cells intravasating from the epithelial compartment into the blood vessel compartment, mimicking early metastatic spread.

A unique advantage of this OOC model is the ability to interrogate how mechanical forces influence cancer cell intravasation. We found peristalsis-like deformations increased the invasion rate of CRC cells, including cell lines and patient-derived colorectal cancer organoids, into the vascular channel. To further understand how peristalsis influences CRC cells, we performed mass-spectrometry based metabolomics on effluent from the channels of CRC-Chips in the presence or absence of mechanical stretching. The differentially expressed metabolites indicated changes to several neurotransmitter levels. Specifically, we determined tumor cells increased secretion of GABA in response to mechanical stretching. Adding exogenous GABA to the epithelial channel was sufficient to increase invasion in the absence of peristalsis. Furthermore, pharmacological inhibition of GABA R_A reduced invasion in the stretched chips, while blocking GABA R_B had no effect on invasion, suggesting a GABA R_A-mediated mechanism. Further insights into how peristalsis increases the metastatic spread of CRC cells through GABAergic signaling may lead to the discovery of novel therapeutic strategies that can halt critical steps in tumor progression.

Presentation: Oral

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Predictive nephrotoxicity testing of ochratoxin A and linkage to CKDu

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Chronic kidney disease of unknown etiology (CKDu) is a condition that is a significant health burden for tropical, developing regions such as Sri Lanka and Central America. The causes of CKDu are unknown; it is thought to involve several environmental factors such as exposure to agrochemicals, heat stress, and ochratoxin A (OTA), a carcinogenic and nephrotoxic mycotoxin found in food products worldwide. Chronic exposure to OTA is unavoidable, and as such, toxicity is a serious concern. Hence, determining the mechanism(s) of OTA-dependent nephrotoxicity may provide key insights into CKDu development and mitigation.

We have developed microphysiological systems (MPS) that were used to identify the specific hepatic enzymes, renal transporters, and intermediate chemical metabolites associated with aristolochic acid mediated CKDu. Cultured under constant flow, primary liver and kidney cells demonstrated localized phase-I/II enzymes and transporters, a significant advance over immortalized cell lines that rapidly lose enzyme expression and transporter polarization.

Our work suggests that OTA may hinder the nuclear translocation of the antioxidant response transcription factor NRF2 from its cytosolic binder KEAP1, thus downregulating numerous oxidative-stress responses regulated by NRF2. GSTP1, a NRF2-regulated enzyme, may be responsible for detoxifying a toxic OTA metabolite pair, ochratoxin quinone/ochratoxin hydroquinone (OTQ/OTHQ), through glutathione conjugation. OTA-mediated antioxidant dysregulation via the NRF2 pathway, specifically GSTP1 downregulation, is hypothesized to facilitate oxidative damage through intracellular accumulation of OTQ/OTHQ.

I will discuss our studies investigating the interactions between OTA, NRF2 agonists and transient heat stress in modulating the OTA-mediated nephrotoxicity using our kidney MPS as well as coupled liver > kidney MPS. A better understanding of the mechanisms of OTA-induced kidney injury will support changes in risk-assessment, regulatory agency policies on allowable exposure levels, and determination of genetic risk factors in high-risk populations.

Presentation: Oral

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A novel approach to interrogating the effects of chemical warfare agent exposure using organ-on-a-chip technology and multiomic analysis

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Organ-on-a-chip platforms are utilized in global bioanalytical and toxicological studies as a way to reduce materials and increase throughput as compared to *in vivo* based experiments. These platforms bridge the infrastructure and regulatory gaps between *in vivo* animal work and human systems, with models that exemplify active biological pathways. In conjunction with the advent of increased capabilities associated with next generation sequencing and mass spectrometry based "-omic" technologies, organ-on-a-chip platforms provide an excellent opportunity to investigate the global changes at multiple biological levels, including the transcriptome, proteome and metabolome. When investigated concurrently, a complete profile of cellular and regulatory perturbations can be characterized following treatment with specific agonists. In this study, global effects were observed and analyzed following liver chip exposure to the chemical warfare agent, VX. The primary mechanism of action of VX is well characterized, and recent studies *in vivo* suggest that VX has additional protein binding partners implicated in metabolism and cellular energetic pathways. However, secondary toxicity associated with peripheral organ systems, especially in human tissues, is not well defined. Our results demonstrate the efficacy of utilizing an organ-on-a-chip platform as a surrogate system to traditional *in vivo* studies by specifically indicating significant dysregulation in response to VX exposure to a number of cellular processes to include but not limited to amino acid synthesis, drug metabolism, and energetics pathways.

Presentation: Poster

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Industry perspective on the challenges and opportunities in developing, selecting and applying advanced *in vitro* models to drug development in the context of data-driven decision making

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Given that the successful translation rate from animal model to clinical trials is limited (can be as low as < 8% as in case of cancer clinical trials), and that toxicities seen in such models often do not correlate to adverse events in patients, we have an increasing and urgent need for the development, refinement and validation of human *in-vitro* models to be used for decision making in pre-clinical safety assessment. There is a rich and fast developing palette of advanced *in-vitro* models that can potentially be used to fill such gaps. Here we outline, from an industry perspective, the main challenges and opportunities we have identified in applying such advanced models to development of safe drugs in the context of data-driven decision-making.

We will discuss the challenges the field faces in terms of model characterization and validation both from the biological and the technical perspective, the factors driving the selection of systems/models available, and the choice, applicability and translatability of readouts. Importantly, this presentation will address the challenges as well as the path forward in the interpretation of multi-model, multi-readout studies, including the need for a data management and data integrity strategy to enable decision making on the short term, and back and forward translatability on the long term.

Presentation: Oral

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The bioengineered intestinal tubule as model to assess biological efficacy of future crops

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Introduction: The ever-growing global population is accompanied by an increase in food demand and decrease in agricultural land. Therefore, innovative solutions to increase crop production are required. Plant breeding is purposed as solution, focused on enhancing yield and disease resilience of crops. However, the effect of genetic alteration of crops on the health of the end consumer is often insufficiently studied. As lettuce is one of the most cultivated crops worldwide, a total of nine different lettuce lines were tested for intestinal biological efficacy.

Method: Bioengineered intestinal tubules were exposed for 24 h to a single lettuce cultivar. Various intestinal parameters were taken into consideration such as intestinal epithelial integrity, cell viability, brush border enzyme activity, inflammatory and oxidation markers. Data obtained were used for a comprehensive cluster analysis to evaluate distinction in intestinal biological behavior between cultivars.

Results: After confirmation of accurate recapitulation of physiological intestinal function, bioengineered intestinal tubules were exposed to different lettuce cultivars. Wild lettuce lines tended to impair the intestinal epithelial integrity after 24 h exposures. Additionally, cell viability, cell attachment and brush border enzyme activity were significantly decreased in wild lettuce lines in comparison to medium control. These detrimental effects were not observed after exposer to domesticated lettuce lines, showing a maintenance of physiological homeostasis. Comprehensive cluster analysis based on all tested biological parameters, revealed clustering of three distinct clusters: (1) commercially available, (2) domesticated and (3) wild lettuce lines.

Conclusion: In this study, a novel *in vitro* model recapitulating the human intestine showed that wild lettuces induced detrimental effects on the intestinal epithelium which gradually decreased as lettuces became more domesticated. This underlines the effect plant breeding can have in the end consumer. Therefore, it is suggested to include biological evaluations in plant breeding, which consequently could aid in breeding towards health promoting crops.

Presentation: Poster

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What are the barriers to adoption? A systematic review of *in vitro* models of gastrointestinal toxicity

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Over the last decade, progress has been made in the development of microphysiological systems (MPS) for absorption, distribution, metabolism, and excretion (ADME) applications. Central to the assessment of new molecular entities (NMEs) for oral applications is the intestine that mediates drug absorption. However, there are limited preclinical methods used to predict absorptive properties of drug candidates available. Moreover, the impact of adverse drug reactions within the GI tract (such as bleeding and diarrhea) is often not well addressed leading to the attrition of the drug during development or post-marketing. Here, we present a systematic review and assessment of the available approaches to study gastrointestinal toxicity (GIT) *in vitro* which provide evidence for scalability and applicability of MPS to drug discovery and development. This approach was selected to ensure that our findings are as objective and reproducible as possible in order to inform best practice for this seeking to validate novel MPS. The search strategy returned a total of 6,088 results and secondary sources contributed another 387 articles. After removal of duplicates (n = 99) a total of 6,376 articles abstracts were screened of which 40 were included for final analysis. A previously published study quality checklist, composed of key insights from regulatory and industrial stakeholders, was utilized to assess MPS models meeting the inclusion criteria. The highly diverse range of cell lines and modelling approaches generated prevented a statistical comparison through a meta-analysis. It was observed that more complex models (n = 11) scored more highly against traditional 2D cell culture models (n = 23) highlighting the potential of such models to predict GIT. from this we have distilled the key outcomes and physiological features required to qualify a novel MPS seeking to address patient variability, mimic the microphysiology of the gut, and accurately predict drug-induced GIT.

Presentation: Poster

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Selection of reliable and reproducible toxicity assays for studying drug hepatotoxicity in a liver-chip

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Numerous toxicity assays are available for *in vitro* studies of drug-induced hepatotoxicity, but the reliability and reproducibility of the results for cells maintained in microphysiological systems (MPSs) need to be evaluated. In our study of acetaminophen hepatotoxicity, we used an Emulate® Liver-Chip, which cocultures primary human hepatocytes (PHHs) with liver non-parenchymal cells (NPCs) consisting of sinusoidal endothelium, Kupffer, and Stellate cells. The Chips are designed such that spent media or effluents for biochemical assays from both channels can be collected at any time point of the experiment. Consequently, we found that three commonly used cytotoxicity assays did not work as expected. Specifically, alanine transaminase (ALT) activity in the PHH effluent spontaneously and remarkably decreased over time making it challenging to determine cytotoxicity using this approach. Such effects were more pronounced in the effluent of acetaminophen-treated PHHs. In contrast, protein levels of ALT were stable for at least 24 h and its elevations in PHH effluent showed good correlations with lactate dehydrogenase (LDH) activity, which accurately reflected cell death. The aspartate aminotransferase (AST) activity behaved similarly to ALT. Although this is not unique to MPS-generated samples, for an LDH activity assay, it was critical to dilute PHH effluent in a stabilizing buffer prior to storage at 4°C or perform the assay with freshly collected effluent samples. Moreover, storing the effluent directly under -80°C without diluting it in the buffer almost eliminated LDH activity in acetaminophen-treated samples. For NPCs, the significant culture medium background prevented the use of an LDH activity assay to determine cytotoxicity, while the activity and protein level of ALT and AST in the effluent were undetectable, even when toxicity severity led to complete cell death. These data highlight the importance of establishing reliable and reproducible toxicity or functional assays for different cell types maintained in MPSs.

Presentation: Poster

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Reproducible brain organoid culture on pillar/perfusion plates for the predictive assessment of developmental neurotoxicity

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Human cerebral organoids represent an innovative direction in predictive developmental neurotoxicity (DNT) screening. However, there are several technical challenges to adopting human brain organoids in preclinical evaluations of drug candidates. Current organoid culture platforms are low-throughput due to tedious manual cell loading and media change and possess batch-to-batch variations affecting the quantitative analysis and reproducibility of results. To address these limitations, we have established high-throughput human cerebral organoid culture in a unique pillar/perfusion plate system built on the footprints of conventional 384-well plates and demonstrated reproducible cerebral organoid culture with minimal manual intervention for the predictive assessment of DNT.

Briefly, iPSCs were seeded in an ultra-low attachment (ULA) 384-well plate to form embryoid bodies which were differentiated into neuroectoderm. The neuroectoderms in the ULA plate were transferred to a 36PillarPlate with Matrigel via a unique sandwiching method which enables robust embedding of neuroectoderms in Matrigel simultaneously without cumbersome manual pipetting. After spheroid transfer, neuroectoderms on the 36PillarPlate were differentiated and matured into cerebral organoids in a 36PerfusionPlate. We compared the growth rates and functions of organoids cultured in both static and dynamic conditions to enhance organoid maturity that are critical for modeling DNT with test compounds. Key biomarkers specific to the neurodevelopmental process such as PAX6, FOXG1, TUBB3, MAP2, and CTIP2 were analyzed and compared. Three replicate experiments were performed at different time points to determine the reproducibility of this method for the cerebral organoid generation with CV below 30%. We further replicated *in vivo* effects of neurotoxic compounds in the developing brain and demonstrated high-throughput, high-content cell function analysis. The miniature cerebral organoid assays developed on the pillar/perfusion plate platform could be implemented to screen and identify chemicals causing DNT thereby restricting the use and limiting the exposure of potentially toxic chemicals to the public.

Presentation: Poster

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InterOrgan multi-tissue chip system for linking matured tissue niches by vascular flow

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Human *in vitro* tissue platforms for studies of integrated human physiology in health and disease are becoming increasingly predictive of clinical data. At present, establishing physiological communication between multiple tissues while preserving their individual phenotypes remains a major challenge that must be overcome to model whole-body physiology and systemic diseases. To this end, we established a scientific premise for developing a bioengineered multi-organ platform for modeling human physiology, that integrates four human organ systems: heart, liver, bone, and skin, all equipped with endothelial barriers and connected by vascular perfusion with circulating immune cells (Ronaldson-Bouchard et al., in press). A major innovative component of the platform is in the biomimetic approach to functional integration, by (i) maintaining a local regulatory niche for each tissue, (ii) connecting tissue compartments by a vascular perfusate containing immune cells, and (iii) establishing a semi-permeable endothelial barrier between the circulatory and tissue compartments. The platform is modular, configurable, PDMS-free and enables real-time imaging and monitoring of cell and tissue functions. Tissues linked by vascular perfusion maintained their molecular, structural, and functional phenotypes over four weeks of culture and showed the expected tissue specific responses during drug screening applications. Multiplexed analysis of complex proteomic data with application of the tools and methodologies from systems biology, physiology and bioengineering highlights the need for maintaining the tissue specific niche during multi-tissue culture to preserve individual tissue maturation over extended timelines. We demonstrated that tissues linked by vascular perfusion preserve individual tissue fidelity, reveal a more clinically relevant PK/PD profile, recapitulated multi-organ toxicity of doxorubicin observed in pediatric and adult clinical study, and enabled identification of clinically relevant early miRNA biomarkers of cardiotoxicity. Overall, the InterOrgan platform can facilitate clinical translation by enabling physiologic communication of phenotypically stable engineered human tissues.

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Reference

Ronaldson-Bouchard et al. (in press). *Nat Biomed Eng*.

Presentation: Oral

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Operating conditions of pillar and perfusion well plates for high-throughput, dynamic 3D cell culture and compound screening

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ug testing and disease modeling on animal models often lack physiological relevance with humans. The 3D tissue models such as spheroids and organoids are being rapidly adopted by academia and the biotech industry to mimic physiological characteristics. However, many of the current organ-on-chip systems (OOCs) are low throughput due to tedious steps for manual cell loading and media change, require cumbersome pump systems to connect different organ modules, and need highly specialized skills and the purchase of expensive proprietary equipment. Developing novel high-throughput platforms implementing physiologically relevant tissue models is extremely critical for large-scale drug screening applications, thereby reducing the preclinical testing costs and replacing the use of *in vivo* animal studies.

To address the limitations that exist in conventional OOCs systems, we have developed an integrated pillar/perfusion plate system for high-throughput culture of spheroids and organoids. In the present work, dynamic cell culture conditions of the pillar/perfusion plates have been optimized experimentally, which was matched with SolidWorks flow simulation. In addition, we have tested whether dynamic cell culture on the pillar/perfusion plates can reduce necrotic core formation, which is crucial in 3D cell models for long-term differentiation and drug screening. ReNcell spheroids with varying seeding density were printed and cultured for 2 weeks to check whether dynamic condition can reduce necrosis in large spheroids. Interestingly, the necrotic core decreased significantly with increased viability in the dynamic culture condition compared to the static culture condition. In conclusion, our platform offered several advantages over contemporary OOCs models, including the use of perfusion well plate without pumps and tubes for long-term cell culture, rapid bioprinting of cells in hydrogels for predictive compound screening, easy collection of samples during the experiment without disturbing the cells, and compatibility with standard 384-well plates and existing high-throughput screening equipment.

Presentation: Poster

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Organismoids – Aiming for ultimate precision in patient-specific treatment selection

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Single- and multi-organ-on-a-chip systems are becoming a sustainable part of the *in vitro* assay landscape for preclinical drug testing. This triggered first attempts to emulate the next level of human biology – miniaturized human bodies on a chip. Autologous organoids of various organs have been differentiated from a single individual donor using induced pluripotent stem cell technologies. These can be used to create miniaturized on-chip bodies. Such models are called organismoids and are supposed to represent miniature, mindless and emotion-free self-contained physiological equivalents of an individual mature human body on a chip (2021, doi:10.3389/fmed.2021.728866). Different terminologies, such as human-on-a-chip, body-on-a-chip or universal physiological template, have been used in the past for an organismoid and it is common sense among the MPS community that organismal homeostasis on chips can be achieved by combining the prime organ equivalents from at least the following ten human systems: circulatory, endocrine, gastrointestinal, immune, integumentary, musculoskeletal, nervous, reproductive, respiratory and urinary. Although ambitious, the development of such human organismoid systems has been recognized as a promising route toward a paradigm shift in the drug development and advancements in precision medicine. The community forecasts at least this decade to establish such functional organismoids on chips (2020, doi:10.14573/altex.2001241). Progress, challenges and visions are discussed in the presentation.

Presentation: Oral

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Development of an arrayed feto-maternal interface (FMi) organ-on-chip (OOC) model for higher throughput assays

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Preterm birth (PTB), or birth before 37th week, is a major pregnancy pathology that contributes to high number of neonatal deaths worldwide. How various molecules, from therapeutics to environmental toxins/toxicants, affect PTB is difficult to study due to limitations in both *in vitro* and *in vivo* models, as well as difficulties on conducting any clinical trials. Microfluidic organ-on-chip systems are great alternative models.

Previously, we have developed a feto-maternal interface (FMi: decidua, amniochorion) organ-on-chip (FMi-OOC) model, composed of four interconnected circular cell culture chambers, which can mimic the structure and functions of the FMi. The model enabled us to create an inflammatory environment with one maternal decidual cell and three different fetal cells and was successfully utilized to study ascending infection as well as the effect of environmental toxicant cadmium on PTB. However, since a single model can only test one condition at a time, a higher throughput model can significantly improve the utility of the FMi-OOC.

In this work, we developed an arrayed FMi-OOC platform that consists of five independent FMi-OOCs on a single chip, where the design is such that a single cell loading step (one of the most time-consuming procedures in OOC testing) can be used to load cells into all five FMi-OOCs through an interconnected microfluidic channel. Importantly, this higher throughput OOC model has a microfluidic delay channel design, which can control the diffusion of fluid so that diffusion occurs between the cell culture chamber within the same OOC structure but not to the neighboring OOC structure even though they are fluidically connected through a microfluidic channel. This design allows the five single FMi-OOCs to be fluidically separated without a much more complex conventional pressure-based valve operation system. Overall, this arrayed FMi-OOC platform can provide a much faster and rapid environmental toxicants screening.

Presentation: Poster

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Development of an innovative cartridge bioreactor for parallelized cultivation and stimulation of complex tissue models

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Musculoskeletal diseases belong to the most common health problems, and the number of affected patients is increasing due to the ageing population. Adequate patient care is only possible if appropriate new therapeutic strategies pass through the preclinical and clinical phases and ultimately reach the market. Particularly in the preclinical phase, the use of animal models is still relied upon, as sufficiently suitable *in vitro* models are not yet available.

In order to close this gap, we successfully developed a preclinical 3D *in vitro* model simulating the initial phase of fracture healing by co-cultivating fracture hematoma models with bone models which consist of self-assembled mesenchymal stromal cells for up to 48 h in a yet static environment. Although we could already demonstrate a similar gene expression and response to the osteogenic inducer deferoxamine as compared to *ex vivo* and *in vivo* data, our preclinical 3D *in vitro* model still lacks features such as mechanical loading and perfusion, which are well-known to influence fracture healing.

Therefore, we are currently developing a modular system based on cartridges integrated in a bioreactor enabling us to cultivate multiple 3D *in vitro* models in parallel simulating the initial phase of fracture healing under well-controlled conditions (temperature, pH, load, perfusion rate). The resulting innovative cartridge bioreactor will allow us to closely mimic the *in vivo* situation of fracture healing and its disorders but also other bone related diseases, biomaterials and other therapeutic strategies as well as the impact of extreme gravitational forces in terms of mechanical stimuli. This will enable us to extend the cultivation time in order to study the transition towards the anti-inflammatory phase. Ultimately, we will gain a sophisticated multidimensional analytic cartridge-based bioreactor suitable to integrate models of bone and other units of the musculoskeletal system including muscles, tendons and joints.

Presentation: Poster

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Perfused Organ Panel™ microphysiological system with synthetic hemoglobin, blood substitute, builds confidence in mitochondrial and xenobiotic metabolism of 3D liver models

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Oxygen is the key to energy production and Phase I xenobiotic metabolism in mammals. It is the final electron acceptor in the mitochondrial electron transport chain providing the bulk of cell energy through oxidative phosphorylation. Many reactive metabolites are formed by cytochrome CYP450 oxidation of drugs and chemicals. CYP450 monooxygenases are membrane-bound heme proteins that use molecular oxygen to catalyze over 95% of intracellular oxidation reactions and the oxidation of exogenous chemicals. Oxygen is essential for catalytic activity of oxygenases, redox reactions, energy production, and the function of ATP-dependent transporters, all of which contribute to human-relevant cell and chemical metabolism *in vitro*. In humans, over 98% of oxygen is delivered to tissues by dissociation from hemoglobin contained in erythrocytes. Only 2% of oxygen is delivered dissolved in blood plasma. Without hemoglobin, oxygen availability for tissues *in vivo* and *in vitro* is low due to its low solubility in blood plasma and culture media. This may contribute to cellular de-differentiation, loss of xenobiotic competence, and the emergence of glycolytic phenotypes that can evade reactive metabolites and free radicals misrepresenting potential *in vivo* toxicity. To address these challenges, Lena Biosciences developed Perfused Organ Panel™ microphysiological system with a synthetic hemoglobin, Blood Substitute. The platform restored oxidative phosphorylation, the dominant mode of energy production in normal cells *in vivo*, providing 4x higher oxygen consumption rate in HepG2 cells without any evidence of oxidative stress compared to 2D. Significantly higher respiratory metabolism for identification of mitochondrial liabilities and significantly higher CYP450 activity (log-fold higher in primary cells) was shown in diverse liver models, including primary human hepatocytes, primary mouse hepatocytes, differentiated HepaRG cells, iPSC-derived hepatocytes, and HepG2 cells cultured in the platform. Perfused Organ Panel™ MPS builds mitochondrial and xenobiotic metabolism confidence in 3D models for human-relevant toxicity testing *in vitro*.

Presentation: Oral

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Classical complement pathway inhibition in a "human-on-a-chip" model of autoimmune demyelinating neuropathies

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Chronic autoimmune demyelinating neuropathies are a group of rare neuromuscular disorders with complex, poorly characterized etiology. Here we describe the development of a phenotypic, human-on-a-chip (HoaC) electrical conduction model of two rare autoimmune demyelinating neuropathies, chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN), and explore the efficacy of TNT005, a monoclonal antibody inhibitor of the classical complement pathway. Patient sera was shown to contain anti-GM1 IgM and IgG antibodies capable of binding to human primary Schwann cells and induced pluripotent stem cell derived motoneurons. Patient autoantibody binding was sufficient to activate the classical complement pathway resulting in the detection of C3b and C5b-9 deposits. A HoaC model, using a microelectrode array with directed axonal outgrowth over the electrodes treated with patient sera, exhibited reductions in motoneuron action potential frequency and conduction velocity. TNT005 rescued the serum-induced complement deposition and functional deficits while treatment with an isotype control antibody had no rescue effect. These data indicate that complement activation by serum from CIDP and MMN patients is sufficient to mimic neurophysiological features of each disease and that complement inhibition with TNT005 was sufficient to rescue these pathological effects and provide efficacy data for an investigational new drug application (#NCT04658472).

Presentation: Oral

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Quality enhancing measures in organ model research

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Organ models are promising new approaches providing unique opportunities for the study of human diseases and treatment designs. However, proving the reliability and trustworthiness of this novel technology will be crucial for its broad acceptance and successful application in translational biomedicine. The aim of the *QUEST center for responsible research* is to establish quality standards in organ model research based on data integrity principles and good research practices in order to enhance research reproducibility. Here, we report on quality measures currently being established at the *Einstein Center 3R*, a recently founded, Berlin based consortium of experts in the development of various organ models. Our first aims are to increase methodological rigor and transparency by collaborative protocol standardization and publication, including the development of minimum reporting standards and structured component testing in organ model research. By implementing these strategies, we envision an increase in the internal validity of different organ model systems. In the next phase, we will investigate practices used for the adoption of organ models in external laboratories. Analyzing the transferability of individual organ models will enable us to evaluate their external validity, map out challenges, define boundary conditions and develop alternate dissemination strategies.

Overall, we aim to establish a modular quality assurance system for *in vitro* discovery science, where the frequency of application can be tailored to the individual research phase of the project – accommodating experimental pipelines and guaranteeing innovation and creativity.

Presentation: Oral

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Development of a functional sarcopenia model on a micro-cantilever platform for drug development efficacy evaluation

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Skeletal muscle physiology is adversely affected by inflammatory diseases and aging. Sarcopenia, a skeletal muscle condition marked with reduced muscle mass and function, is often observed as a comorbidity with inflammatory disorders. One of the approaches to studying sarcopenia is to develop a defined model system evaluating skeletal muscle force dynamics on a micro-cantilever platform and then, to combine it with multi-organ micro-physiological devices to study sarcopenia induced by an inflammatory response from other organs. Here, we have developed a defined *in vitro* functional model system to study sarcopenia by incubating human induced-pluripotent stem cell-derived skeletal muscle cells in 3nM TNF-alpha during differentiation. We have extensively evaluated the long-term effects of this treatment up to 32 days in culture. To assess disease phenotype, we quantified myotube width, myotube contraction amplitude, fatigue index, and force readout upon integration into the micro-cantilever system. Our model revealed a significant reduction in myotube width up to 32 days in the TNF-alpha-induced sarcopenic condition. In addition, we recorded a decrease in myotube contraction amplitude and an increase in the fatigue index for 32 days by characterizing muscle contractions through phase differential analysis. On silicon-based micro-cantilever systems, induction of sarcopenia led to consistently lower contractile force for up to 28 days. Disease progression and tissue degradation was monitored which indicated sarcopenic conditions had higher ROS production for both acute (day 4) and chronic (day 40) timelines. Overall, this model evaluated sarcopenia progression based on muscle mass, function, and strength and is being employed for drug development efficacy studies.

Presentation: Poster

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Analysis of rat iPSC-cortical neurons on MEA and its comparison with human iPSC-cortical neurons

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For the last several decades, one of the most prevalent models for preclinical research has been the rodent model. However, as previous clinical trials have shown, efficacy in rat models can be poor predictors for drug efficacy in humans. In this context, human induced pluripotent stem cells combined with human-on-a-chip technology presents a microphysiological platform for more sophisticated analysis of neuronal networks. This technology could allow for the obsolescence of most animal testing as a more accurate predictor of efficacy in humans, while simultaneously reducing cost and time. Induced pluripotent stem cell (iPSC)-based modeling also provides an achievable bridge to translate rodent-based preclinical findings to human systems. We have developed a human iPSC (hiPSC) cortical neuron-MEA system that is able to analyze long term potentiation (LTP), the cellular base of learning and memory. This study sought to develop a rat iPSC (riPSC)-derived cortical neuron-MEA model and compare it with a human platform for their functional characteristics and drug sensitivity. Phase microscopy was first used to monitor morphology of riPSC cortical neurons throughout differentiation and maturation. Cortical neuron differentiation and network formation was characterized and confirmed through the expression of a group of markers (Nestin, Ctip, MAP2, and Synaptophysin etc.) using immunocytochemistry. The electrophysiological characteristics of the riPSC-cortical neurons was analyzed by whole-cell patch-clamp at multiple time points. Neurons were further analyzed utilizing microelectrode arrays (MEAs) for their electrical activity and their capability to reproduce LTP. The sensitivity to multiple drugs was characterized utilizing the LTP assay and compared with those from the hiPSC-cortical neuron system. Comparison between rat and human iPSC-derived cortical neuron systems will shed light on the species gap between rat and human, which would facilitate the translation of preclinical data collected from rat to clinic applications.

Presentation: Poster

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Microphysiological systems in medical countermeasure development

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The Biomedical Advanced Research and Development Authority (BARDA) supports the development and procurement of medical countermeasures (MCM) – to address CBRN threats, pandemic influenza and emerging infectious diseases. Since advanced microphysiological systems (MPS) are enabling technologies for studying human biological responses (e.g., pathophysiological/immune responses, host-pathogen interactions), BARDA has included MPS as a major component in its research and development portfolio. BARDA is exploring the use of MPS in accelerating drug development and rapidly identifying therapeutic candidates in a health emergency.

BARDA, through its Division of Research, Innovation, and Ventures (DRIVe), is also investing in more general efforts to enhance MPS usability and position it as an integral component of the drug development process. BARDA is prioritizing automated manufacturing solutions, making this technology easier to access and disseminate. Further, DRIVe is focusing on enhancing validated human tissue models with immune system components and incorporating biochemical sensors for long-term tissue monitoring. By integrating advances along these three lines of effort, BARDA aims to enable widespread adoption and translation of MPS technology.

BARDA's Chemical, Biological, Radiological and Nuclear Countermeasures Division is investing in several programs to advance these technologies. The Chemical Medical Countermeasures branch has focused on the development of human lung models to examine the effects of chlorine inhalation, leading to identification of new therapeutic targets for drug repurposing. The MPS will also be used for drug screening. In the future, these lung MPS can accelerate the development of countermeasures against emerging inhalational threats.

The Radiological and Nuclear Countermeasures branch looks to bring MPS programs into the portfolio to elucidate the natural history of irradiation injury. Pathophysiologies of interest include systemic cellular apoptosis, endothelial cell damage, altered cell metabolism, and immune dysregulation. Our ability to understand and treat these conditions could be greatly enhanced with MPS platforms.

Presentation: Poster

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Functional neuronal platform to investigate pathology in iPSC-cortical neurons carrying PSEN1 and APP AD mutations

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Alzheimer's disease (AD) can onset as a result of familial AD (FAD) mutations of the presenilin 1 (PSEN1) or amyloid precursor protein (APP) genes. The downstream effects of these mutations give rise to abnormal cleavage of APP into amyloidβ peptide 42 (Ab42). The accumulation of Ab42 forms destructive amyloid oligomers, fibrils and plaques which cause synaptic dysfunction. Drug development for AD has encountered many obstacles and a significant one is the lack of human-based functional models, which causes difficulties for preclinical to clinical translation and thus a high failure rate during clinical trials. Microphysiological systems containing microelectrode arrays (MEAs) make it possible to develop accurate and efficient preclinical models capable of predicting the effects of therapeutics. A human induced pluripotent stem cell (hiPSC) MEA model capable of generating functional long-term potentiation (LTP) has been established and validated, furthermore, this model lends itself the ability to observe the functional phenotype of neural networks in a FAD hiPSC models. For this study, we characterized the FAD PSEN1 and APP mutant hiPSC-CNs to identify AD-relevant pathologies by comparing to their isogenic controls. Phase microscopy was used to monitor morphological differences throughout 40 days of differentiation and immunocytochemistry utilizing markers of ctip2 and MAP2 confirmed successful differentiation of cortical neurons while staining of synaptic puncta indicated synaptic deterioration over time. Patch-clamp electrophysiology was also utilized to characterize the electrical properties of the differentiated neurons. Neural network activity formed by patterned iPSC-CNs was evaluated on MEAs to analyze their basic electrical activity and the induction and maintenance of LTP, as LTP is a correlate for learning and memory. The ability to construct these AD models and effectively compare their functional and characteristic behavior allows for further applications such as screening of current and novel treatments for AD.

Presentation: Poster

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Differential monocyte actuation in a three-organ functional innate immune system-on-a-chip

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Hesperos has developed a pumpless, serum-free multi-organ system featuring recirculating immune cells with cardiomyocytes, skeletal muscle, and liver in separate compartments (Sasserath et al., 2020). This platform allows for investigations into targeted immune responses and tissue-specific damage, along with holistic organ responses to inflammatory compound exposure. The targeted response featured fluorescently labeled THP-1 monocytes selectively infiltrating an amiodarone-damaged cardiac module and changes in contractile force measurements without immune-activated damage to other organ compartments. In contrast, general proinflammatory treatment of circulating immune cells with lipopolysaccharide (LPS) and interferon-γ (INF-γ) causes nonselective damage to cells in all three-organ compartments. Biomarker analysis indicates upregulation of inflammatory cytokines TNF-α, IL-6, IL-10, MIP-1, MCP-1, and RANTES following LPS+INFγ treatment, whilst amiodarone treatment leads to an increase in IL-6. These results were compared to those obtained with primary monocytes in the platforms to understand and contrast to the THP activation studies. Taken together, these data highlight the potential for this system to serve as a more functionally relevant alternative to humanized animal models for determining the direct immunological effects of biological therapeutics such as monoclonal antibodies, vaccines, and gene therapies; or, with determining the indirect downstream effects resulting from a target tissue response to a drug's pharmacokinetics (PK)/pharmacodynamics (PD) profile.

Reference

Sasserath, T. S., Rumsey, J. W., McAleer, C. W. et al. (2020). Differential monocyte actuation in a three-organ functional innate immune system-on-a-chip. *Adv Sci 7*, 2000323.

Presentation: Poster

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Development of a human malaria-on-a-chip disease model for drug efficacy and off-target toxicity evaluation

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The parasite, *Plasmodium falciparum*, is the pathogen responsible for the disease known as Malaria. Over the decades many anti-malarial therapies have been developed, and more are still in development to eradicate this persistent global disease. One of the primary roadblocks to the development of anti-malarial treatments is the lack of good *in vitro* model systems that can reproduce the reproductive life cycle of *Plasmodium falciparum*. A functional, human, multi-organ, serum-free, pumpless system was fabricated to reproduce the erythrocyte replication cycle and this microphysiological system introduces innovative techniques in therapeutic drug development. The platform contains 4 human organ constructs that include primary human hepatocytes, primary human splenocytes, human umbilical vein endothelial cells (HUVECs), as well as recirculating primary human red blood cells which allow for the infection and dispersion of the *P. falciparum* parasite for both a chloroquine responsive and chloroquine resistant strains. The maintenance of viable functional cells was successfully demonstrated both in healthy and diseased conditions for 7 days in this recirculating microfluidic model. To demonstrate that the system can provide an effective platform for malaria therapeutic compound development, systems were acutely treated with the anti-malarial compound, chloroquine. Parasite levels were significantly reduced in a dose dependent manner and parasite recrudescence was observed by the fifth day for the chloroquine responsive strain and only minimally responsive to the resistant strain. This system also evaluated the off-target toxicity of the anti-malarial treatment in a dose dependent manner highlighting the ability of these multi-organ MPS devices to determine therapeutic index for anti-malarial therapeutics. The work performed establishes a new approach for the evaluation of anti-malarial therapeutics in a realistic human model with recirculating blood cells.

Presentation: Poster

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Chronic vs acute drug evaluation in a multi-organ human-on-a chip preclinical toxicity testing platform

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One of the key questions that is addressed by animal experiments during pre-clinical drug discovery and for other toxicological investigations is the reaction to repeat doses over longer periods of time for drug candidates or other compounds. A significant majority of current *in vitro* models focus only on acute, bolus doses of a compound that is monitored for a short duration and reported as an administered dose in the literature. What is needed are systems that can not only accurately describe acute dosing in terms of compound exposure to an organ mimic but also repeat dosing or chronic administration as it's a more accurate reflection of real-world exposure profiles of interest to clinical toxicologists. Multi-organ human-on-a-chip systems provide a unique platform to address this gap in testing ability that could provide significant data to understand dose profiles at the pre-animal stage of testing, but also if human cells are utilized, could be used to help predict outcomes for clinical trials. Here we describe a human multi-organ MPS device incorporating non-invasive functional readouts to monitor the effects of both acute and chronic dosing. These data are combined with HPLC derived pharmacokinetic data of drug compound concentration profiles for prediction of first in human maximum tolerable dose (MTD) and the no observed adverse effect level (NOAEL).

Presentation: Poster

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A patient-derived lung-on-chip model for immunotherapy safety assessment

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Folate-receptor 1 (FOLR1) is overexpressed in various types of cancer (ovarian, lung, renal), making it an attractive candidate for targeted-tumor immunotherapy. Latest advances led to development of T-cell bispecific antibodies engineered to recognize both FOLR1 and the T-cell receptor CD3 (FOLR1-TCB), harnessing the immune system for tumor recognition and killing. Despite its innovative potential and efficacy, since FOLR1 is also expressed in healthy epithelial cells in the lung or the kidney, this immunotherapy presents the risk of on-target/off-tumor pulmonary toxicity (Kerns et al., 2021), which must be investigated.

In this work, the AlveoliX lung-on-chip technology was employed to investigating FOLR1-TCB alveolar toxicity *in-vitro* (Stucki, Hobi et al., 2018). With that purpose, FOLR1-TCB was administered to human epithelial and/or endothelial barrier models in the presence of peripheral blood mononuclear cells (PBMC). According to our results, this treatment induced a pronounced on-target/off-tumor damage in the alveolar model as indicated by increased cytotoxicity, barrier leakage (TER) and pro-inflammatory cytokine release (e.g., IL-6, Granzyme B). Additionally, FOLR1-TCB treatment induced recruitment of immune cells to the epithelium, as detected by live imaging tracking, as well as T-cell specific activation, as observed by flow cytometry analysis.

Taken together, our results suggest that our patient-derived alveolar lung-on-chip model can successfully predict off-tumor TCB adverse effects and is therefore a suitable non-animal tool for safety evaluation of therapeutic antibodies in preclinical studies. This work highlights the relevance of employing human-specific immunocompetent 3D *in-vitro* models, allowing cell-cell interaction, and resembling the *in-vivo* like microenvironment for an accurate clinical predictability. Moreover, access to patient data such as clinical history and pathological screening of tissue, creates a unique connection between the clinics and *in-vitro* studies, opening the route towards personalized medicine and relevant translational outcomes.

Presentation: Oral

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A 3D-optimized microplate enables spheroid production, long-term cultivation, and confocal high content imaging with cell-level resolution in a single plate

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Homeostasis at the tissue level is maintained through finely orchestrated cell-cell and paracrine signaling which frequently involves several different cell types. The cyto-architecture of tissues, including the organization and the ratio of native cell types, plays an important role in maintaining normal tissue function and viability. Studying individual cell responses in the context of intact tissue is therefore critical to understanding the development, prevention, and reversal of human disease. To that end, we have developed multi-cellular 3D liver, islet, and tumor microtissue models (a.k.a. spheroids), and an accompanying Akura™ 384 microplate technology that supports microtissue production, cultivation, and high-resolution 3D imaging. The combined platform enables the cell-level analysis of spheroids with added spatial/architectural context. The platform is also scalable and automation-compatible making it ideal for studying tissue-level responses at early stages in the drug discovery pipeline.

Using pancreatic islet microtissues we examined the compatibility of the Akura™ 384 spheroid plate with confocal high-resolution imaging. Human islet microtissues, reconstituted from dissociated native islets, were labeled with two nuclear markers, DAPI and anti-NKX6.1 (beta cell-specific marker). Using a 3D nuclear colocalization assay requiring single cell resolution, we compared the images acquired from 3D microtissues in a Akura™ 384 plates to those acquired in a u-bottom spheroid plate. Our results demonstrate that a continuous, flat, ultra-thin, transparent bottom significantly minimizes the refractive index mismatch and chromatic registration issues observed with u-bottom plates and enhances the overall speed and accuracy of the image acquisition.

As the dependence on 3D models and high content imaging continues to expand, maximizing 3D image quality is imperative to the development of accurate and comprehensive spheroid measurements. Innovations, such as the development of standardized 3D models and 3D-optimized microplates, may soon overcome the remaining obstacles limiting the broader utilization 3D cell models in early drug discovery.

Presentation: Oral

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A breathing, immortalized lung-on-chip cell model: An optimized tool for robust drug testing and inhalation toxicology at the alveolar setting

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Complex *in vitro* lung models are increasingly becoming a key asset in the drug development pipeline, disease modeling and inhalation toxicology. Modeling the distal airway, though, has proven relatively challenging, and thus the standardization of reliable alveolar *in vitro* models has not yet been achieved. These limitations include scarcity of tissue, donor-to-donor variability, spontaneous differentiation on cell culture supports, and the difficulties of recapitulating the dynamic alveolar microenvironment.

In this work, we describe a novel and reproducible lung *in vitro* model by combining a primary-derived immortalized alveolar epithelial cell line (^{AX}iAEC) with the ^{AX}Lung-on-chip technology, which replicates outmost physiological conditions: (1) breathing-like cyclic stretch (10% linear strain, 0.2 Hz) and (2) an ultrathin, porous and elastic membrane.

Our results showed that this cell line is characterized by the coexistence of cells consistent with AT1- (HTI-56+, Cav-1+) and AT2-like (SP-C+, HTII-280+) phenotypes. Alveolar and barrier markers were upregulated over time, and further accentuated by the introduction of air-liquid interface (ALI) and breathing conditions. Cells also achieved robust barrier formation (TER > 1000 Ohm cm^2) in ALI up to 21 days, and in co-culture with endothelial cells, leading to the formation a tight air-blood *in vitro* barrier that could be used for long term studies.

Finally, we challenged the model using profibrotic (TGF-β) and proinflammatory (LPS) factors to test its suitability to model tissue repair, lung inflammation and fibrosis. Both TGF-β and LPS, led to a weakening the barrier function and the expression of EMT-associated genes, and secretion of proinflammatory cytokines (IL-8), respectively.

Our immortalized alveolar model on-chip expresses key characteristics of the *in vivo* alveolar epithelium in conditions such as those of the alveolar microenvironment (breathing, ALI, co-culture). This model is hence a promising tool for robust *in vitro* testing for different molecules including safety, efficacy and toxicology in the distal lung.

Presentation: Oral

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Investigate the effect of hyperglycemia on muscle development and function utilizing human iPSC-derived model

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Diabetic myopathy, a physical complication of the more commonly known diabetes mellitus, is characterized by reduced muscle function such as muscle atrophy and can be modeled using a hyperglycemic media system. In this study, we sought to investigate the direct effect of hyperglycemia on muscle development in a human induced pluripotent stem cell (hiPSC)-derived skeletal muscle culture system. HiPSC myoblasts were first expanded in media with varying concentrations of glucose at 5.55 mM (physiological level), 17.5 mM, and 25 mM. It was observed that myoblasts cultured at higher concentrations of glucose had slower proliferation. The myoblasts were then differentiated into myotubes in the same media across all conditions. There was a notable difference in myotube fusion, with hyperfusion and branching observed via phase microscopy and immunocytochemistry in myotubes that were pre-cultured in the higher glucose conditions. To evaluate muscle contractile function, a microphysiological system involving stimulation with a bath electrode and pixel subtraction of a region of interest was employed. At day 7 and day 10 of maturation, the muscle was stimulated using multiple frequencies to assess the contractile phenotype. In higher glucose conditions, the myotubes fatigued earlier compared to the physiological glucose condition. These results were further validated against primary satellite cells. The findings directly demonstrate the negative effect of hyperglycemia on skeletal muscle development and function. The establishment of this disease model enables further exploration of this disease and potential treatments for functional recovery.

Presentation: Poster

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2D intestinal crypt platform enables evaluation of compound effect on stem cell proliferation and differentiation

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The intestine is the most common site of adverse drug reactions in clinical trials, and there is a significant need for improved model systems for compound screening. To address limitations of existing *in vitro* systems, we are developing a 2D, self-renewing intestinal model (Raehyun et al., 2018), termed the 2DCrypt. The 2DCrypt platform is based on a standard SBS plate format, and is populated with human colonic stem cells which organize and develop into spatially segregated stem and differentiated cell compartments. The planar geometry of the 2DCrypt systems easily supports dosing and sampling, as well as high-throughput imaging. Mature 2D crypts replicate many aspects of *in vivo* physiology including cell migration and maturation between stem and differentiated compartments and apoptosis at inter-crypt boundaries. We have we established an immunofluorescence-based assay, enabling us to quantitatively assess cell viability, proliferative cell abundance and migration, the abundance and localization of all post-mitotic lineages as a function of time, and impaired cell survival/culture longevity. Using this approach, we have evaluated crypt spacing and geometry modifications to enhance culture longevity, and present data to establish the 2DCrypt as a system in equilibrium. We have used the 2DCrypt platform to perform a small-scale screen of compounds known to impact proliferation and/or differentiation including signaling molecules, endogenous hormones/cytokines, and microbial metabolites. We report statistically significant increases and decreases in the relative population of proliferative cells, goblet cells, and colonocytes following a 5-day dosing regimen. The 2DCrypt platform represents a significant innovation in the development of physiologically relevant microphysiological systems, as it is the first established model which is spatially organized into self-sustaining crypts.

Reference

Raehyun, K. et al. (2018). Formation of arrays of planar, murine, intestinal crypts possessing a stem/proliferative cell compartment and differentiated cell zone. *Lab Chip 18*, 2202-2213.

Presentation: Poster

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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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Rodent derived synthetic blood brain barrier model for chemical toxicity screening

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Exposures to environmental toxins such as pesticides, heavy metals (e.g., Hg) and high levels of essential metals (e.g., Mn) leads to pathological conditions including inflammation, oxidative stress eventually leading to neurodegenerative diseases. Although significant discoveries have been made using isolated cells cultured under static environments, lack of physiological relevant *in vitro* models has hampered the understanding of key mechanisms and how exposure to metals alters the BBB.

In this study, we have developed a novel microfluidics based vascularized rodent BBB model which enables mechanistic understanding and functional analysis following exposure to toxins and high levels of essential metals. This model was developed using co-culture of endothelial cells, astrocytes and neurons followed by detailed characterization for biomarkers and tight junction formation using real-time permeability analysis. Essential metal toxicity was investigated by varying Mn dosage and pesticide toxicity was investigated using Cypermethrin. Cellular viability was assessed using Live/Dead assay while permeability was monitored using 4kDa FITC-dextran. Results indicated both a dose and culture dependent response. Viability decreased as concentration was raised and an increase in permeability was observed. In addition, significant difference was observed between mono-culture model comprising individual cells and the tri-culture model suggesting the importance of the interaction occurring between the cellular components of the BBB.

Future studies include validation against *in vivo* animal models and development of diseased conditions following interactions with toxins. The developed assay will have critical applications in basic research for understanding of chemical toxicity mechanisms and development of toxin neutralization strategies.

Presentation: Poster

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A multi-scale model of the osteochondral unit

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Osteoarthritis (OA) is the most common degenerative joint disease and represents the leading cause of chronic pain in the industrialized world. OA affects over 32 million adults in the U.S. alone. Despite its prevalence, treatments of the disease are limited to pain management and surgery. There are no effective pharmacological interventions to slow down or even reverse the disease, in part because of the poor understanding of the underlying etiological mechanisms of OA. Increasing evidence points to a significant role of the interplay between subchondral bone and articular cartilage, which highlights the urgent demand for realistic 3D models of the osteochondral unit. To address this need, we have developed a multi-scale, multi-cellular 3D osteochondral model that includes cartilage, subchondral bone, and vasculature.

We have developed a multi-scale *in vitro* platform comprising of a microfluidic device for rapid screening and a meso-scale bioreactor to mimic *in vivo* scale conditions. Our tissue models integrate osseous, chondral, and vascular components in communication with one another and permits both real time on chip and off chip analyses to investigate cell-cell interaction between tissue compartments. We successfully differentiated patient derived human mesenchymal stem cells towards osteogenic and chondrogenic lineages and highlighted interactions with vascular endothelial cells, as demonstrated by immunohistochemistry, histological staining, gene expression, and fluorescent assays. Following this detailed functional characterization, we demonstrated the capability of our platform to evaluate functionality for anti-inflammatory therapeutics.

This platform represents the first integrated microphysiological system of the vascularized osteochondral unit and will permit the fundamental study of OA and its mechanisms and drug development studies to identify potential therapeutics.

Presentation: Poster

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Comparison of MSC derived extracellular vesicles on PDMS-free lung-cancer-on-a-chip and lung-on-a-chip systems

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Extracellular vesicles (EV) have been shown to modulate various intercellular communications in our bodies, including therapeutic effects of mesenchymal stromal cell (MSC) EVs. Recent studies show that MSC EVs have a tumor suppressor effect. Also, MSC EVs have been proposed as a targeted cancer therapy delivery tool in their natural form or modified and loaded with drugs. However, most of the EV studies are done either on static 2D or 3D cell cultures or in animal models. The 2D and 3D cultures often lack relevant biological barrier, vasculature, and flow interactions. Additionally, one of the major problem for organ-on-a-chip broader application is the main material polydimethylsiloxane (PDMS), which does allow rapid device prototyping, but it absorbs small fat soluble molecules and small lipid particles, which is not suitable for EV delivery research. Here we use Lung-on-a-chip devices that are made from a hybrid thermoplastic that has significantly lower small molecule and lipid particle absorption. Currently we are comparing MSC EV effects on lung-cancer-on-a-chip made from A549 cells and on healthy primary cell lung-on-a-chip and we are also comparing these with transwell models.

Presentation: Poster

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Characterization of variability in primary human intestinal cell cultures

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Cells are a key component of microphysiological systems; primary cells more faithfully represent *in vivo* physiology and open the door to personalized medicine. However, establishing commercial quality metrics or even establishing quantitative bounds of acceptable cell behavior constituting reproducibility has proven difficult for primary cell cultures. Reproducibility across experiments is essential for credible, informative research and is a necessity for commercial applications. Our aim was to characterize consistency across human intestinal stem cell (HISC) lots generated from the transverse colon of multiple donors, as cultured in the RepliGut© Planar culture system. We have evaluated cell performance across 3 donors, using $n \geq 3$ lots per donor and $n \geq 2$ culture runs per lot. Metrics for reproducibility included: (1) cellular growth kinetics, (2) functional measures of epithelial barrier formation and maintenance, and (3) immunohistochemical assessment of the proportion of all intestinal post mitotic lineages in fully differentiated RepliGut© cultures. Furthermore, we characterized the dose-dependent pharmacology of TNFα, highly relevant to inflammatory bowel disease, across donors and lots, establishing ranges for the IC50 and maximum effect size. Our findings show distinct donor to donor variability, as would be expected given genetic and biologic variability. However, we were able to quantitatively establish reproducibility within and between cell lots for our transverse colon cells. Our characterization of cell behavior at baseline and in response to insult will facilitate improved experimental design and improved assay quality.

Presentation: Poster

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High content contractile analysis of microphysiological 3D skeletal muscle model using a magnet-based noninvasive real-time platform

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Engineered muscle tissue (EMT) has been developed as an *in vitro* model for highly efficient physiological and pharmaceutical studies. In this study, we developed a physiologically relevant EMT model that allows for magnet-based real-time noninvasive contractile analysis suitable for disease modeling and drug screening. These EMTs were generated by self-assembly of skeletal myoblasts in collagen/Matrigel composite hydrogel formed around two polydimethylsiloxanes (PDMS) posts, one rigid and one soft. Magnets pre-loaded onto the tip of the soft posts allow for magnet-based real-time quantification of EMT contraction. Our data showed that the EMTs could develop optimal twitch forces at 20V, 1 Hz. while they showed tetanic forces at 20V, 20 Hz, with a tetanic to twitch ratio of 2.4 on Day 5 and 1.9 on Day 10 after tissue casting. EMTs produced contraction in response to electrical stimulations at frequencies increasing from 1 Hz to 20 Hz. Single twitch turned into repetitive, synchronous twitches as frequency increased from 1 Hz to 5 Hz and finally generated persistent tetanic force at 20 Hz. Long-term culture and periodic contractile analysis showed a continuous increase in tetanic and twitch force. Maximal tetanic (0.61 mN) and twitch force (0.35 mN) were observed on Day 10. Whole-mount immunohistochemical staining results showed a mature, striated structure of contractile protein sarcomeric α-actinin (SAA) and F-actin. Myotube length and cross-sectional diameter were analyzed based on SAA-staining results, which showed an average diameter at 10.12 μm and an average length at 316.25 μm for EMTs on Day 5. The development of this EMT model will potentially lead to a high-throughput screening assay, which will enable us to test the toxicity/efficacy of novel drugs at the preclinical phase.

Presentation: Poster

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Optimizing microphysiological systems for operating in Biosafety Level 3 and 4 spaces

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Design should follow function. Microphysiological systems (MPS) must first recreate the physiology of the system they seek to model. They must outperform the standard cell culture paradigms and ideally be a better representation of human physiology than our current animal models. However, with the rise of the COVID-19 global pandemic, when data generation needed to happen at warp speed and experiments must be run in the highly restricted space of a Biosafety Level 3 (BSL-3) laboratory, new or redesigned and optimized chips and systems are required to accommodate these additional considerations. Studies with more virulent pathogens could require BSL-4 containment, which places even stronger restrictions on removal of samples and hardware from the controlled space. Operations in both BSL-3 and BSL-4 are greatly simplified if the entire MPS system is fully contained, does not require any mechanical hardware such as pumps or valves, and is disposable and even incinerable. In this collaborative project we have created a new two-compartment airway chip and redesigned our existing, two-compartment neurovascular unit (NVU) / blood-brain barrier (BBB) chip to be simpler to use, have greater throughput, and to meet both the space and containment restrictions of either a BSL-3 or BSL-4 lab, thus extending the capabilities of MPS. Our new devices have large yet shallow, gravity-fed reservoirs that can provide for 24 hours the high flow rates required to polarize the BBB in the NVU, readily form an air-liquid interface for the airway chip, are fully enclosed by a secondary container, and are still compatible with high-content imaging. While the current designs are fabricated from PDMS, they could be readily fabricated by injection molding from polystyrene or cyclic olefin copolymer. We present results on exposure of both chips to SARS-CoV-2.

This work was supported in part by NIH NCATS award 3UH3TR002097-04S1.

Presentation: Poster

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Assessing contractility of 3D iPSC-derived muscle models for safety and discovery using a novel, high-throughput, and label-free instrumentation platform

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Stem cell models hold great promise for improving the predictive power of preclinical *in vitro* assays for new therapies, drug discovery, and disease modeling. Complex 3D platforms, such as Engineered Muscle Tissues (EMTs) fabricated from primary or iPSC-derived cardiac and skeletal muscle cells, can directly measure tissue contractility. However, traditional methods to fabricate EMTs demand extensive bioengineering expertise; measuring contractility often involves laborious, serial, and low-throughput optical measurements.

We report on the design, fabrication, and validation of a novel 3D EMT platform that uses 1) facile and scalable approaches to generate tissues from a variety of cell sources, and 2) a label-free parallel measurement technique. Our tissue casting approach has a success rate of > 96% (n > 1,000), produces consistently-sized constructs, and is highly amenable to automation.

The substrate features an embedded magnet; as tissues contract, the magnet's displacement is quantitatively detected in a highly-parallel manner using specialized sensors. We detected 24 contractions simultaneously with a measurement rate of 100 Hz, which is suitable for measuring various aspects of contractility such as upstroke velocity, decay time, and fatigability.

We will present data showing acute effects of drugs measured minutes after EMT dosing and chronic effects of structural cardiotoxicants like doxorubicin, sunitinib, and BMS-986094. All chronically-dosed tissues showed statistically significant dose-dependent reduction in twitch frequency over a multi-day time course ($p < 0.05$).

In addition to modeling healthy tissues, the platform can also be used to study disease models. We are currently developing patient-specific Duchenne Muscular Dystrophy models for the development of personalized gene therapies. EMTs can be made from cells sourced from patients and used to test the effects of new therapies.

The platform will provide a standalone tool capable of screening significant numbers of compounds for rapid safety and efficacy evaluation of drug candidates, thereby accelerating drug discovery and development.

Presentation: Oral

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Alk1-deficient endothelial cells drive vascular malformation in a microphysiological disease model of hereditary hemorrhagic telangiectasia

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Hereditary Hemorrhagic Telangiectasia (HHT) is a rare congenital disease that affects 1 in 5000 births, and in which mutations in endothelial-expressed genes such as *ACVRL1* (Alk1) drive the appearance of vascular malformations (VM) including microvascular overgrowths (telangiectasias) and arteriovenous shunts. These VM affect blood vessels of the liver, brain, and skin, and rupture of these aberrant vessel structures significantly compromises patient quality-of-life and can even be fatal. There is currently no cure for HHT, and efforts to develop such treatments are challenged by mouse models that do not appear to completely recapitulate the pathophysiology of HHT in humans, as well as the lack of available *in vitro* models that use human cells and that can mimic the microenvironment in which healthy and diseased blood vessels form. Here we present a microfluidic vascularized micro-organ (VMO) system wherein application of fluid flow induces primary human EC and co-seeded perivascular cells to spontaneously self-organize into a perfused blood vessel network. Inclusion of stromal cells from brain (astrocytes, neurons) or liver (hepatocytes) produces a VMO that exhibits specific characteristics of these respective tissues. Using the VMO platform, we show that primary human EC, engineered (via RNA silencing) to lack *ACVRL1* (Alk1) expression, form aberrant blood vessel networks, including structures reminiscent of both the telangiectasias and arteriovenous shunts typically seen in HHT patients. We also show compatibility of the VMO-HHT platform with HHT patient-derived cells. Lastly, we find that VM that form in the VMO-HHT platform are normalized following exposure to pazopanib, a putative HHT drug. Taken together, we describe a robust, scalable tissue-specific microphysiological disease model of HHT that we expect will support further studies into the pathophysiology of HHT as well as significantly improve the drug discovery and testing pipeline for HHT patients.

Presentation: Poster

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Development of a functional human iPSC-cortical neuron-MEA model for long term potentiation analysis and Alzheimer's drug testing

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Alzheimer's disease (AD) is commonly associated with a characteristic loss of the cognitive functions associated with memory and learning due to synaptic decline caused by the improper cleavage of amyloid precursor protein. Long-term potentiation (LTP) has been utilized as a means of assessing synaptic plasticity by evaluating the functional response of neuronal networks to high-frequency stimulation as LTP is a correlate for learning and memory. Our study aimed to develop a human-based AD model for the evaluation of cognitive integrity by reproducing this pathology on patterned microelectrodes. The use of multi-electrode arrays (MEAs) with the surface patterned using photolithographic chemistry enabled synaptic development between paired populations of neurons. By integrating a previously published human iPSC-cortical neuron culture onto these MEAs, a synapse-driven model of LTP was validated for biologically relevant evaluation of synaptic function. Synaptic plasticity was assessed in each neuronal pair by stimulating one neuronal population and analyzing the connected population for synapse-mediated response and the induction of LTP. LTP induction was confirmed by AMPA and NMDA receptor blocking experiments, which demonstrated the mediation of synapse-mediated responses and/or the maintenance of persistent LTP. This system was further adapted for modeling amyloid pathology by administering Ab42 oligomers to healthy cortical neurons. Neurons dosed with Ab42 oligomers experienced an inability to maintain LTP for 1 hour. Neuronal damage from a 24-hour acute dosage of Ab42 was also assessed through whole-cell patch clamp recordings. This functional AD model was validated for drug testing by co-administration of four current AD therapeutic treatments. With the treatments, there was no loss of neuronal function in patch clamp recordings, and the synaptic integrity was sustained in the ability of the neurons to maintain persistent LTP. Thus, we have established and validated a biologically relevant, human-on-a-chip model of AD capable of assessing preclinical drug effects.

Presentation: Oral

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Positive inotropic and chronotropic effects of rapamycin in the I-Wire human cardiac tissue construct

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Mechanistic target of rapamycin (mTOR), a kinase that plays a vital role in the regulation of growth, differentiation, and metabolic processes (Brown et al., 1994), inhibits mTOR by interacting with the cytosolic FK506-binding protein of the 12 kDa-rapamycin complex (Sciarretta et al., 2014). 3D engineered cardiac tissue constructs (ECTCs) present a unique possibility to conduct pharmacological and toxicological studies and model cardiac function and diseases *in vitro*. We investigated the effect of rapamycin on mechano-elastic properties and conducted transcriptome analysis in our I-Wire cardiac constructs (Sidorov et al., 2017). Human induced pluripotent stem cells and fibrin-based extracellular matrix were utilized to differentiate cardiomyocytes in molds and grow ECTCs. Measurements of contractility and elasticity were conducted at differentiation days 28-32 using protocols we developed. Our experimental platform is based on an inverted microscope and records small deflections of the flexible probe by CMOS camera (Sidorov et al., 2017). The transcriptome was analyzed using RNA-Seq, and statistically significant gene groups were determined via gene set enrichment analysis (GSEA). Treatment with 10 nM of rapamycin for 2 days caused a positive inotropic effect in force-tension relationship with from 21.6% to 28.2% for applied tensions of 0.32 mN to 0.74 mN, respectively. The spontaneous ECTC beating accelerated from 0.92 ± 0.22 Hz to 1.11 ± 0.25 Hz. Gene set enrichment analysis was conducted based on annotation of three categories and two databases: biological process, molecular function, cellular component, KEGG and WikiPathways. The representative categories were primarily related to development, proliferation, and cell death in downregulated genes and to lipid metabolism in upregulated genes. Cardiomyocyte differentiation in molds shortens the time to grow ECTCs and allows mechano-elastic measurements after three weeks of the differentiation protocol. The observed positive inotropic and chronotropic effects of rapamycin are related to stimulation of lipid metabolism and maturation of the ECTCs.

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Presentation: Poster

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An automated microphysiological system for pharmacokinetic and -dynamic studies with paper-based cultures

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Bringing a drug to market is a costly and time-intensive process, with only one in ten drugs successfully completing clinical trials. This high failure rate is due, partially, to the inability of current *in vitro* and animal models to predict human responses. The 2 and 2.5-dimensional cultures currently used in pharmacokinetic and -dynamic (PK/PD) evaluations do not represent the physiological conditions and cellular microenvironments present *in vivo*. To better predict *in vivo* responses, and thus reduce the cost and time required to bring a drug candidate to market, 3D microphysiological systems (MPS) are needed in the drug discovery workflow. Here, we developed and validated a low-cost and modular MPS capable of performing automated PK/PD studies with 3D paper-based cultures supporting colorectal cells individually and in co-culture with hepatocytes. This 3D paper-based system can support mono- and co-cultures for at least 72 h. We demonstrate this MPS system's ability to perform PD evaluations of cytotoxicity by sampling the culture medium at 1 h intervals to determine cellular viability in the presence of chemotherapies (e.g., continuous and dynamic exposures of SN-38 at 10 μM and 10^{-3} μM, respectively). The dynamic exposures are used to mimic the excretion of chemotherapies from the body, with a half-life of approximately 40 h. In addition, we demonstrate the ability to perform PK studies through quantifying the conversion of the chemotherapy Irinotecan to SN-38 at 1 h intervals with an in-lab validated LC-MS/MS method. This new device provides a starting point for the inclusion of non-traditional culture platforms into the drug discovery workflow. It also demonstrates the utility of paper-based cultures and the small technological barrier of incorporating them into MPS systems.

Presentation: Poster

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A multiscale computational framework for modeling microphysiological systems

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Over the past few decades, the use of mathematical models to support various stages of drug development and to predict cellular behavior has seen significant increase. Of particular interest is the effective use of pharmacokinetic/pharmacodynamic-toxicity (PK/PD-Tox) models for dose optimization, toxicity threshold identification, translation of *in vitro* compound potency to the *in vivo* setting, reduction in the number of *in vivo* studies and improvement of results translation from preclinical species into the clinical setting. The majority of these models use simplified lumped compartments to simulate *in vitro* drug transport, absorption, distribution, metabolism and excretion. This approach uses ordinary differential equations with non-physiological parameters to represent complex biological processes and tissues, which may fail to adequately capture relevant gradients or zonation in the *in vitro* environment. Alternatively, high-fidelity first principles-based models that use spatially resolved geometries and multiphysics approaches to describe species transport and elimination are able to capture these gradients, but are computationally expensive. In microfluidic organ-on-chip systems, use of high-fidelity models may be more appropriate design for analyzing flow patterns, pressure drops, wall shear stress profiles, membrane mechanical loads, etc.; however, reduced-order models are more suitable for modeling long-term drug transport, and PK/PD-Tox effects. Here we present and demonstrate a multiscale modeling approach combining lumped and spatially resolved first principles-based mathematical models for capturing the intricate biophysical details captured by microphysiological systems. These models have been used to i) maximize chip performance (i.e., optimize flow rate to achieve physiologically relevant shear stress and adequate oxygen/nutrient distribution across the system), ii) identify/describe underlying mechanisms to elucidate the PK-PD relationship (i.e., production of toxic byproducts that induce cell death) and iii) support *in vitro-in vivo* translation (i.e., scaling metabolism from chip to human). Examples of validated results highlighting different aspects of the framework will be presented.

Presentation: Oral

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Tissue engineered cancer microsphere production for high-throughput screening

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Three-dimensional (3D) spheroidal models have been established as more effective than two-dimensional models in collecting drug response information. However, self-aggregated tumor spheroids lack control over extracellular matrix components and exhibit heterogeneity in shape, size, and aggregate forming tendencies. Here, we overcome these challenges by coupling tissue engineering toolsets with microfluidic technologies to create engineered cancer microspheres.

Specifically, we employed biosynthetic hydrogels composed of conjugated poly(ethylene glycol) and fibrinogen protein to create engineered breast and colorectal cancer microspheres for 3D culture, tumorigenic characterization, and examination of potential for high-throughput screening (HTS). MCF7 and MDA-MB-231 cell lines were used to create breast cancer microspheres and the HT29 cell line and cells from a stage II patient-derived xenograft (PDX) were encapsulated to produce colorectal cancer (CRC) microspheres.

Using our microfluidic system, highly uniform cancer microspheres (intra-batch coefficient of variation (CV) $\leq$ 5%, inter-batch CV < 2%) with high cell densities (> 20×10^6 cells/mL) were produced rapidly. Encapsulated cells displayed cell type-specific differences in morphology, proliferation, metabolic activity, ultrastructure, and bulk stiffness. For PDX CRC microspheres, the percentage of human and CRC cells was maintained over time and similar to the original PDX tumor, and the mechanical stiffness also exhibited a similar order of magnitude (10^3 Pa) to the original tumor.

The microspheres were compatible with an automated liquid handling system for administrating drug compounds. MDA-MB-231 microspheres were distributed in 384-well plates and treated with staurosporine and doxorubicin, and expected responses were quantified, demonstrating initial applicability to HTS. PDX CRC microspheres were treated with Fluorouracil and displayed a decreasing trend in metabolic activity with increasing drug concentration. Providing more physiologically relevant tumor microenvironment in a high-throughput and low-cost manner, the hydrogel-based cancer microspheres could potentially improve the translational success of drug candidates by providing more accurate *in vitro* prediction of *in vivo* drug efficacy.

Presentation: Poster

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Modeling skeletal muscle fibrosis and vascular interactions using a human microphysiological system

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Skeletal muscle fibrosis is a clinical condition commonly caused by muscle injury, dystrophy, diabetes, sarcopenia, and rheumatoid arthritis and is characterized by scarring, which impairs muscle contractile function and causes severe pain, affecting an individual's range of motion and quality of life. Muscular fibrosis is attributed to excessive deposition of extracellular matrix (ECM) in the skeletal muscle. We used human skeletal muscle engineered muscle bundles (myobundles) to examine factors which can promote loss of muscle function. We matured myobundles for 7 days, added 1, 5 and 10 ng/mL transforming growth factor beta (TGF-beta) for 3 days, and measured mechanical properties and active contractile force. The tetanus force was significantly reduced at all concentrations of TGF-beta for 3 different donors. The bundle elastic modulus increased at 10 ng/mL TGF-beta for 2 of the 3 donors and higher stiffness was associated with increased production of collagen 1, 4 and 5 and fibronectin determined by RT-PCR and immunofluorescence. Fibroblasts were confined to the periphery and did not increase in number after TGF-beta treatment. An inhibitor (SB525334) of the downstream Smad pathway reduced the inhibitory effect of TGF-beta on force and the increase in collagen production. Next, to evaluate the effect of fibrotic muscle on vascular function, myobundles with or without pre-treated with 5 ng/mL TGF-beta were then connected to TEBVs under perfusion conditions. Under controls conditions, untreated myobundles did not affect TEBV function. In contrast, myobundles pretreated with TGF-beta reduced endothelium dependent vasoconstriction and increased VCAM-1 expression on endothelium. We also observed a trend of increased alpha smooth muscle actin and fibronectin by PCR suggesting an endothelial to mesenchymal transition. These results suggest the muscle fibrosis can affect vascular function.

Presentation: Oral

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Modeling inflammatory immune cell recruitment and response on human colon intestine-chip

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Objective: Immune cell recruitment into tissues is an essential step in inflammatory responses. This occurs in a highly tissue- and stimulus-specific manner, which presents a significant challenge to modeling disease and testing therapeutics *ex vivo*. We previously developed an advanced primary human vascularized Colon Intestine-Chip model and showed that it recapitulates physiologic cell composition, morphology and barrier function. The goal of this study was to test the ability of this system to model inflammatory bowel disease (IBD)-like immune cell reactions *ex vivo*.

Methods: We perfused primary human peripheral blood mononuclear cells (PBMC) across the vascular channel in untreated "resting" or TNFα/chemokine-treated "inflamed" Colon Intestine-Chips. We analyzed total cell recruitment, inflammatory cytokine secretion and barrier function in the following 24-72 hours.

Results: We show that the perfused PBMC efficiently adhered and transmigrated to the epithelial channel in an inflammation-specific manner. This was followed by an accumulation of pro-inflammatory cytokines characteristic of IBD (e.g., INFγ, IL1β, IL18), as well as loss of barrier function, the hallmark feature of IBD. We further showed that 1) the recruited cells were strongly enriched of the "gut trophic" a4b7+/CCR9+ PBMC subsets and 2) this recruitment could be blocked with the IBD therapeutic Entyvio, which targets the a4b7-MAdCAM-1 interaction.

Conclusion: Our findings indicate that our Colon Intestine-Chip can model inflammatory immune cell recruitment and *in situ* immune reactions that reflect key clinical correlates of IBD. This model may prove effective for development of new anti-inflammatory therapeutics for human intestinal diseases.

Presentation: Oral

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A juvenile dermatomyositis model based on Type I interferon interactions with engineered human skeletal muscle

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Upregulation of Type I interferons (IFN I), such as IFN-α and IFN-β, is a hallmark of juvenile dermatomyositis (JDM), but their role in the pathogenesis of the disease is not clearly understood. This study assessed the effect of IFN I on healthy pediatric skeletal muscle using an *in vitro* 3D engineered biomimetic construct. Due to challenges isolating muscle cells from JDM patients, myogenic cells isolated from healthy pediatric donors were cultured and used to create myobundles. IFN-β exposure for 7 days at concentration from 5, 10 and 20 ng/mL was associated with decreased maximum tetanus force of the myobundles. While the twitch force did not show a consistent decline with IFN-β dose, the time to maximum twitch and half-time for relaxation increased with increasing dose. IFN-β exposure was associated with a statistically significant increase in the major histocompatibility complex I (MHC I), the myositis-specific autoantigen MDA5, and the endoplasmic reticulum stress marker GRP78 mean fluorescence in longitudinal sections. No change in the levels of the autoantigen Mi-2 were observed in response to IFN-β exposure. In cross-sectional images only GRP78 showed an increase in mean fluorescence intensity. IFN-β exposure did not have a significant effect on nuclei density, myofiber diameter, or structural proteins dystrophin, sarcomeric alpha-actinin and vimentin. In contrast, myobundles treated with IFN-α for 7 days did not show a changed in tetanus force or twitch kinetics. Although MDA5 did not increase with IFN-α dose, MHC I levels increased with IFN-α dose. The results suggest that this 3D engineered tissue platform is a promising model to further develop for JDM research.

Presentation: Poster

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Electrical pulse stimulation and compounds with anti-atrophic potential influence contractile response of patient-derived skeletal muscle cells in a microphysiological system

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Muscle wasting leads to a dramatic decline in mobility in an increasing aging population, yet age-related muscle loss or sarcopenia remains without effective therapeutic options. Microphysiological systems or organs-on-chips have the potential to advance screening of drugs to counteract pathologies *in vitro* with clinically relevant physiology. This work applies a human muscle-on-chip that enables functional testing of donor-derived myobundles with electrical stimulation with the long term goal to identify effective exercise mimicking regimes and therapeutic compounds against sarcopenia. Cells isolated from biopsies of the vastus lateralis muscle of adult donors were encapsulated into a collagen-Matrigel hydrogel and injected into a PDMS chip designed to promote cell alignment while a surrounding channel facilitated diffusion of culture medium. Tissue chips were perfused with growth medium for 2 days, then after 12 days of intermittent perfusion with differentiation-conducive media, electrical pulse stimulation was applied via platinum wires embedded in the chip. Finite element analyses of fluid dynamics and electric currents within the conductive media were examined in COMSOL Multiphysics® to predict the electrical field strength sensed by the cells based on applied voltage. Images were acquired at 10 frames per second to track tissue contractions upon stimulation and the resultant videos were analyzed in an established MATLAB® algorithm. Simulations showed a homogeneous electric field when 3V were applied at the electrodes and video analysis confirmed a ten-fold increase in average contractions when bundles were stimulated twice a day by electrical pulses for a week. Evidence of significant average contractions, peak displacement amplitudes, and relaxation displacement were obtained from chips that received stimulation twice daily compared to once daily. Differences in contraction parameters were also observed with increasing anti-atrophy compound doses, which demonstrates a successful implementation of a muscle-on-chip for the study of tissue response to therapeutic treatments proposed to counteract muscle wasting.

Presentation: Oral

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Utilizing organs-on-chips to fill human health data gaps on exposure to per-and polyfluoroalkyl substances

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Per and polyfluoroalkyl substances (PFAS) are persistent and pervasive chemicals frequently encountered by people and wildlife. As a chemical class, PFAS include some 4000 different compounds making it difficult to adequately assess human risks due to PFAS exposure. As such, there are a number of data gaps which often times cannot be filled through traditional means for assessing exposure outcomes such as two-dimensional tissue culture on plastic, or *in vivo* models. Organ-on-a-chip models and other full tissue models, have become widely adopted as more accurate means for predicting human outcomes in response to exposure to drugs or compounds of interest. The organ chips developed by Emulate offer a two channel design comprised of multiple cell types that can interact with in their microenvironment as they would in a human organ system. The chips are subjected to constant, unilateral flow, which can be useful to both facilitate exposures from exogenous compounds and provide the cells with the sheer stresses comparable to what would be experienced *in vivo*. In this study, Emulate liver and renal proximal tubule chips were exposed to various concentrations of PFOS or PFHxS over time. Cytotoxic effects, which were different from those observed in 2D tissue culture, were detected as a result of exposures to both compounds. Phenotypes that may be detrimental to organ health after exposure to PFOS or PFHxS were also identified, such as changes in uric acid, albumin production and ROS/RNS production compared to untreated chips. PFOS and PFHxS were detected in cell lysates and medium effluent over time, providing an outlook on the pharmacodynamics properties of the compounds. These data highlight organs-on-chips as a viable means for generating predictions on the effects of PFAS compounds on human health.

Presentation: Poster

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Pillar and perfusion plate platforms for robust organoid culture and analysis

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Human organoids have potential to revolutionize *in vitro* disease modeling by providing multicellular architecture and function that are similar to *in vivo*. This innovative technology, however, still suffers from assay throughput and reproducibility to enable high-throughput screening (HTS) of compounds due to cumbersome organoid differentiation processes and difficulty in scale-up. Using organoids for HTS is further challenged by lack of easy-to-use fluidic systems that are compatible with organoids. Here, we overcome these challenges by engineering "microarray three-dimensional (3D) bioprinting" technology and associated pillar/perfusion plate platforms for human organoid culture and analysis. We have successfully manufactured the pillar/perfusion plates via injection molding of polystyrene and demonstrated static and dynamic human liver and intestine organoid culture with functional assays. The pillar/perfusion plates maintained long-term organoid culture with bioprinted cell suspension and spheroids transferred to the pillar plate with Matrigel by supporting either static culture with growth media in the deep well plate or dynamic culture with a flow of growth media through the perfusion well plate without the use of pumps. Developed cell printing and encapsulation protocols were highly flexible and allowed for culturing multiple organoids on the pillar plate, consequently providing more insight into potential organ-specific toxicity of compounds. Our microarray 3D bioprinting technology demonstrated on the pillar/perfusion plates represent a unique strategy of printing organoids in biomimetic hydrogels on the pillar plate rapidly. Human organoids on the pillar/perfusion plate platforms could recapitulate tissue development and maintain tissue functions by mimicking *in vivo* microenvironments. Additionally, mechanistic actions of compounds could be elucidated *in vitro* through high-throughput, high-content analysis. We envision that human organoids on the pillar/perfusion plates could provide highly predictive toxicity and efficacy information needed in preclinical evaluations of compounds. Thus, our unique approach could offer wide industrial adoption of organoid-based assays for HTS.

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Evaluation of usefulness of coculture with MPS by drug efficacy test using kinetic-pump integrated microfluidic plate (KIM-Plate)

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Microphysiological systems (MPS) are a novel evaluation system for pharmacokinetics, pharmacodynamics, and toxicity in drug discovery. In Japan, the Japan Agency for Medical Research and Development (AMED) has been conducting an MPS development project aimed at the practical application of MPS. Our research group has developed a Kinetic pump Integrated Microfluidic Plate (KIM-Plate), for commercialization in collaboration with participating Japanese companies.

The KIM-Plate is a novel multi-organ MPS platform integrated with stirrer-based kinetic pumps for easy evaluation of organ-to-organ interactions in cell-based assays (Shinha et al., 2021). The KIM-Plate has a simple structure consisting of open-type 24-well size cell culture chambers connected by microchannels. The greatest advantage is that it can easily coculture highly conditioned cells, because commercially available culture inserts and cell disks can be used in cell preparation.

We performed a drug efficacy test on the KIM-Plate to evaluate the usefulness of coculture using MPS. Human hepatoma model cells derived from chimeric mice and cancer model cells were cocultured on the KIM-plate to evaluate the efficacy of prodrug-type anticancer drugs. In addition, the conditioned medium method, in which cancer model cells are cultured in the same culture medium used for hepatocyte culture, was used as a conventional assay system for drug efficacy testing. The results showed that the coculture on the KIM-Plate showed higher drug efficacy than the conditioned medium method. This result suggested that the conditioned medium method may underestimate drug efficacy because it does not reproduce changes in drug concentration due to metabolism.

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 KIM-Plate and details of the drug efficacy study.

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Modeling glia population towards physiological ratios and functionality in human brain organoids

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The exquisite functionality of the human brain stems in part from the complex interplay between neuron and glia cells, thus approximating physiological cellular composition is crucial in modeling the human brain. Oligodendrocytes and astrocytes are key players in setting the ground for proper form and function of the brain. Astroglia support neuron metabolism by providing mitochondria and neurotransmitter components to neurons. They are instrumental for the migration, positioning, and maintenance of a functional environment for neurons, and consequently, enhance myelination. Their absence is associated with disorganized white matter architecture, loss of blood-brain barrier integrity, and dysmyelination. Glia presence is necessary for debris clearance and pruning of synapses in biological learning as they refine and sustain patterns of brain connectivity and plasticity. Furthermore, astrocytes are immunocompetent cells that respond to challenging environments.

Although brain organoids provide a relevant platform for studying human biology and neurological diseases, they have not yet acquired physiologically relevant ratios of neuron to glia cells, which according to recent calculations is close to 1:1. We optimized our previously published protocol (Pamies et al., 2017) of brain organoids to trigger glia differentiation without compromising neuronal differentiation. Two induced pluripotent and two embryonic stem cell lines were used to compare outcomes. Using qPCR, flow cytometry, confocal imaging and electron microscopy, we found that the number of oligodendrocytes increased by 20%, and astrocytes over 40%, potentially enhancing electrical conductivity and synapse plasticity. Astrocytes also changed the morphology in glia-enriched medium, better recapitulating primary culture astrocytes. We did not observe significant changes in neurons as seen by gene expression and neurite outgrowth assay. Our results have the potential of improving *in vivo*-like functionality of brain organoids for the study of neurological disease and drug discovery, contributing to the unmet need for safe, human-model drug development.

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Streamlining 3D high content-imaging by combining automated 3D spheroid culturing, staining and imaging in a multifunctional well-plate

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High-content imaging using three-dimensional tissue structures involves a series of sequential steps, each of which requires optimization to achieve maximal output with minimal technical variability. 3D spheroid models have gained interest due to their physiological relevance, including their 3D architecture and inclusion of relevant cell types. Further, their scalability and compatibility with 96 and 384 wellplate formats makes them ideal for higher-throughput studies. For suspended spheroids, all steps leading up to high-content imaging require adaptation; including liquid handling protocols for efficient medium exchange without tissue loss, staining protocols preserving tissue morphology while ensuring dye penetration, and selection of imaging compatible cultureware to enable high-resolution fluorescence imaging.

We have previously demonstrated that InSphero's Akura™384 enables adherent-free spheroid culturing and minimal tissue loss even when 90% of the volume is removed. This function is enabled by an integrated well-ledge that protects the microtissue from accidental aspiration. We took advantage of this design to implement a fully automated staining protocol. The protocol includes fixation, permeabilization, and antibody staining of the microtissues with multiple washing steps. The protocol can be run autonomously overnight (15 h). Automation of steps with long incubation times significantly reduces the time cost of the sample preparation and prevents manual pipetting errors.

GFP-expressing tumor spheroids were stained with DAPI (nuclei) and anti-beta integrin (cell surface receptor). The stained spheroids were imaged using Yokogawa's CQ1 high-content system. The microplate comprises a 25 µm-thin flat Teflon bottom-foil and a 1-mm wide observation window. The thin bottom enables high-quality *in-situ* imaging with high NA short working distance objectives, even for larger 3D spheroids. The acquisition of a complete 384-well plate for 3 fluorescent channels, 7 z-slices took 93 min. We were able to image the GFP (cytosol), DAPI (nucleus) and the beta-integrin (cell membrane) to demonstrate imaging of different cellular parts.

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PREDICT96: Demonstrating high-throughput diverse complex tissue, therapeutic efficacy screening, and biomarker identification

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PREDICT96 is the highest throughput (96 independent samples on one standard size microplate) organ-on-chip (OOC) system in existence which readily interfaces with industry-standard infrastructure and data collection tools. Active and precise pumping of each 96 individual OOC device, each containing two microchannels separated by a thin membrane, is controlled by 192 individual micropumps contained within the plate lid, and there are also 384 electrical contacts to make real-time electrical measurements/stimulation. PREDICT96 has been optimized for minimal secreted factor and drug dilution and sorption and is enabled for high-content microscopy and virtually any biological assay.

Here we report on several new and impactful applications of this system for replicating complex barrier tissue biology at scale. First, we will detail PREDICT-ALI, our human primary upper airway model which was the first OOC model to demonstrate robust SARS-CoV-2 infection and viral replication. We evaluated unique infection kinetic profiles across a diversity of human donors in our BSL3 facility using multiple assays enabled by the 96 device/plate throughput of PREDICT96-ALI. We also investigated the antiviral efficacy of many compounds, including Remdesivir and MPro61, against SARS-CoV-2 infection, correlating to clinical results. Second, we will detail how we used our PREDICT96-Alveolar platform to evaluate specific exosome therapies within an acute respiratory distress syndrome (ARDS) model and correlate to *in vivo* data. Finally, we will show how our PREDICT96-vasculature model was utilized to identify new biomarkers of drug-induced vascular injury (DIVI). After generating disease human and rat disease models, we compared *in vitro* and *in vivo*/clinical trends in rats and humans, cross-species trends, and ultimately identified potential robust translational DIVI biomarkers. Collectively, these case studies demonstrate the flexibility and tenability of PREDICT96 to create physiological-relevant biology at high-throughput scale for evaluation of therapeutics in BSL2 and BSL3 spaces.

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A microphysiological system coupling of metabolic associated fatty liver disease (MAFLD) to endocrine pancreatic islets: Towards the association and causal link between MAFLD and type 2 diabetes (T2D)

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Western diet can lead to excess circulatory free fatty acids (FFAs) that promote hepatic lipid droplet formation and dysregulation of FFA oxidation. In the absence of lifestyle modification, this initially reversible hepatosteatotic state remains. With the onset of MAFLD, hepatic and then systemic insulin resistance develops (Gough et al., 2021). Despite systemic insulin resistance, hyperinsulinemia due to β-cell compensation forestalls hyperglycemia. Progression to type 2 diabetes (T2D) occurs with eventual β-cell failure. Mechanisms underlying β-cell failure and the role of hepatokines in this pathophysiology remain poorly understood due to a complex interplay of organ cross-talk.

Microphysiological systems (MPS) allow for modulatory examination of organ-specific events through the control of complex biological architectures and spatial-temporal trials (Gough et al., 2021). Our vascular Liver Acinus Microphysiological System (vLAMPS) recapitulates the hepatic endothelial and parenchymal space through four cell-types culture, liver specific ECM, and flow-induced portocentral zonation (Gough et al., 2021). In parallel, a vascular Pancreatic Islet (vPANIS) MPS was developed to mimic a vascular bed and a parenchymal space to culture primary human pancreatic islets. In the integrated platform we have established an oxygen gradient at the liver parenchyma level while retaining primary islet function of Glucose Stimulated Insulin Secretion (GSIS) with a common flowing medium.

Islet GSIS evaluations were used to fine-tune the chemically defined basal common media between liver and pancreas, Normal fasting media (NFM). NFM perfused in a two-organ system, showed sustained viability of human islets and expression of β-cell specific C-peptide, while maintaining glucose-dependent insulin secretion over 2 weeks of culture. It also supported primary human hepatocytes with a minimum level of steatosis. The media was further customized to recapitulate early metabolic syndrome (EMS) leading to steatosis, nonalcoholic steatohepatitis, fibrosis, and insulin resistance. One-week, upstream vLAMPS with a downstream vPANIS exposure to EMS leads to loss of GSIS, as well as reduced expression of C-peptide. Our modular MPS platform allows a systemic communication that is facilitating intermediary inquiring between organs, enabling the identification of MAFLD exclusive hepatokines that may promote downstream loss of β cell function.

Reference

Gough, A. et al. (2021). Human biomimetic liver microphysiology systems in drug development and precision medicine. *Nat Rev Gastroenterol Hepatol 18*, 252-268.

Presentation: Oral

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Functionally enigmatic genes in neurodegenerative diseases: Using mini-brains to illuminate human specific genes in the CNS

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Previous research has shown that pathway or annotation based gene analysis – a mainstay of most bioinformatics pipelines – is biased by overlooking both primate-specific genes and genes involved in complex disease phenotypes, as compared to genes with a Mendelian inheritance pattern. This presents a particular problem for neurodegenerative disease, as they typically involve many genes with subtle effects on the phenotype as well as several genes which may be primate-specific, or serve different functions in the primate CNS compared to rodent models – one reason why animal models for neurodegenerative diseases have typically performed poorly. Here, we show that transcriptomic data from mini-brains can help illuminate many genes that are important in neurodegenerative processes that have been overlooked from the literature. In addition, we show that relying on pipelines focusing only on annotated genes will likely overlook many important genes.

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Microphysiological systems to interrogate the islet-liver-adipose axis in normal physiology and type-2 diabetes mellitus

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Diabetes and associated chronic diseases and complications remain one of the biggest challenges to public health. Yet, few effective treatment options for obesity-driven inflammation and insulin resistance are available. To address this pressing concern, we are developing interconnected microphysiological systems (MPS) using human pluripotent stem cell (iPSC or ESC)-derived metabolic tissues for glucose and fatty acid regulation (liver and fat) and insulin secretion (islets) in conjunction with an immune component (macrophages). Toward the goal of utilizing a unified cell source, three mature and hormonally responsive tissues were generated from the iPSCs. Adipocytes expressed hallmark genes at levels that match or exceed those found in primary adipocytes and exhibited robust responsiveness regarding isoproterenol and insulin. Hepatocytes showed insulin-induced reduction and glucagon-stimulated elevation of glucose production. Macrophages showed pro-inflammatory cytokine patterns comparable to primary cells. ESC-derived beta-cell clusters (islets) responded to physiological glucose stimulation with 2-3-fold increase in insulin/C-peptide secretion. Coculture conditions have been established for fat, liver and macrophages. In the liver-MPS, pro-inflammatory M1 but not anti-inflammatory M2 macrophages caused insulin resistance that greatly reduced insulin effectiveness to suppress hepatic glucose production. Macrophages introduced into the fat-MPS migrated into the 3D adipose tissue over time and formed crown-like structures around dead adipocytes, recreating critical features of adipose architecture seen *in vivo*. Again, M1 but not M2 macrophages induced insulin resistance and dysregulated lipolysis in adipocytes. We further established conditions for the interconnection of fat and liver-MPS. Using fluorescently labeled fatty acids preloaded into adipocytes, we could demonstrate lipid transfer from the fat to the liver-MPS. Importantly, dysregulated lipolysis in fat-MPS containing M1 macrophages caused insulin resistance in the liver-MPS, thereby recapitulating key features of obesity-associated fatty liver disease. Current experiments are underway to integrate fat, liver and islet-MPS to recapitulate glucolipotoxicity, a hallmark of type-2 diabetes mellitus progression.

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Multiorgan microphysiological systems as tools to understand interorgan crosstalk in health and disease

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Multiorgan microphysiological systems (MOMPS), mimicking complex human physiology, offer new possibilities to provide clarity in the origins of systemic metabolic and inflammatory diseases. Controlled interaction of individual human tissues and the scalability of biological complexity in MOMPS, supported by advances in systems biology, might hold the key to identifying novel relationships between interorgan crosstalk, metabolism, and immunity. The Trapecar lab is integrating 3D bioprinted donor-matched tissues into MOMPS to investigate how i) interorgan communication directs complex tissue development and organ-level renewal and how ii) a disruption thereof leads to the emergence of immunometabolic pathologies. We show that tissue-level interaction between and within the three main germ layers ectoderm (neurons), mesoderm (lymphoid), and endoderm (gut and liver) leads to increased tissue maturation and increased *in vivo*-like functionality. In our approach, we reconstruct donor-matched hepatic, gut-mucosal, and neuronal tissue via digital light-assisted bioprinting, under fluidic communication and the presence of the donors circulating immune cells. We compare tissue maturation, longevity, and functionality across interacting versus isolated tissue and identify major contributors to altered performance via proximity protein labeling. We further use the established system to derive how a disruption in immune-tissue signaling contributes to overlapping inflammatory disorders of the gut-liver axis. Paired with computational tools and resolution into molecular underpinnings of cellular and tissue homeostasis, MOMPS represent a unique opportunity to systematically dissect how interactions at a lower order inform new behavior at the macroscale within and between organ systems. Such scalable complexity might yield new insight into causal relationships between our genetic markup, the environment, our life choices, and the fundamental emergence of disease.

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Development of a humanized *in vitro* vascular model to study hypercytokinemia and endothelial dysfunction in SARS-CoV-2 infection

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/COVID-19) has resulted in over 5.6 million deaths to date. High mortality COVID-19 is associated with a hyperaggressive inflammatory response characterized by hypercytokinemia and endothelial damage. Recent advances in vaccination practices for SARS-CoV-2 have reduced hospitalization rates, but hypercytokinemia remains a major concern among those with breakthrough infections leading to severe COVID-19. There are still gaps in our knowledge regarding the exact mechanisms in the pathology of this hyperinflammatory response; here we present a disease model that recapitulates this COVID-19 hypercytokinemia to investigate these molecular pathways. We have previously developed a Vascularized Micro Organ (VMO) microphysiological system that supports *de novo* formation of a perfused and physiological microvasculature. Here, we adapt the VMO to model endothelial dysfunction following SARS-CoV-2 infection. We find that expression of renin-angiotensin signaling axis components, which mediate SARS-CoV-2 cell entry, is upregulated in endothelial cells in the VMO compared to 2D monolayer. Additionally, we show that SARS-CoV-2 pseudovirus has increased infectivity in the VMO compared to in monolayer, and that exposure of the VMO to SARS-CoV-2 pseudovirus reduces ACE2 expression while increasing key pro-inflammatory markers. Perfusion of recombinant Angiotensin II (rAng II) in the VMO mimics SARS-CoV-2 infection suggesting enhanced renin-angiotensin signaling contributes to endothelial dysfunction in COVID-19. To mimic widespread organ dysfunction we utilized effluent from the pseudovirus treated VMO to demonstrate the disseminated effects of COVID-19 infection on our pancreatic islet based platform and subsequent dysregulation glucose metabolism.

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NerveSim platform for translational neurological drug discovery and toxicology research

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Microphysiological systems are rapidly being adopted as advanced *in vitro* tools for modeling neurological disease, development, and injury, and as drug discovery platforms. Neurological drug development is particularly fraught with failures as the animal models used in preclinical studies do not translate well to human biology in most neurological indications and with evolving modern drug modalities. Here we present the only *in vitro* peripheral nerve model with Schwann cell myelination and with the ability to perform nerve conduction and advanced excitability studies on our proprietary embedded electrode array (EEA) electrophysiology platform. We developed and commercialized this 3D Nerve-on-a-Chip for drug screening that enables both physiological and histological evaluation in manners analogous to clinical evaluation. We have further developed methods for assembling multiple neural tissue types and shown the potential of this approach for modeling the transmission of physiological signals associated with pain. Recent efforts have been focused on the integration of these tissue systems with integrated microelectrode technology to enhance our ability to investigate the electrophysiological behavior of these model systems. This model has been applied in research of drug-induced peripheral neuropathy (DIPN), amyotrophic lateral sclerosis (ALS), and pain with plans for additional peripheral and central nervous system disease indications across nearly all drug modalities.

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Biomaterial hiPSC encapsulation improves cardiomyocyte differentiation yield with enhanced functionality in suspension culture

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Although suspension-based, scalable production of cardiomyocytes (CMs) has typically employed self-aggregated embryoid bodies (EBs), encapsulation of human induced pluripotent stem cells (hiPSCs) in hydrogels provides tighter control over the cellular microenvironment. Employing a microfluidic system to rapidly encapsulate hiPSCs within PEG-fibrinogen microspheres (MS), here we compared the MS differentiation and resulting cardiac tissue function to suspension-differentiated EBs. MS were more consistent in size and shape. Resulting MS had an initial diameter of 673 ± 22 µm (coefficient of variance, CV = 0.03) and axial ratio of 1.03 ± 0.01 (CV = 0.01), while EBs had an initial diameter of 145 ± 34 µm (CV = 0.24) and axial ratio of 1.15 ± 0.04 (CV = 0.03). MS had a significantly higher number of CMs (2.8 ± 1.8 versus 0.6 ± 0.12) at day 10 per initial hiPSC and higher CM yield (75 ± 14% CMs versus 56 ± 1 6%) compared to EBs (cTnT+, MF20+, $n \geq 5$, $p < 0.05$). Myocardial contractility was also enhanced in MS compared to EBs (day 10); maximum contraction velocity was 4x higher for MS than EBs (139 ± 17.5 versus 33.4 ± 5.1 µm/s) and maximum relaxation velocity was 14x higher (97 ± 7.2 versus 7.4 ± 0.4 µm/s). The capacity of hiPSC-CMs to appropriately respond to isoproterenol (IP) and propranolol (P) pharmacological stimulation was compared. Microspheres significantly responded (frequency increased 124% (IP) and decreased 61% (P)), whereas for aggregates changes in frequency were less pronounced (frequency increased 49% (IP) and decreased 27% (P)). These results suggest that microsphere hiPSC-CM β-adrenergic signaling may be more mature. Calcium transient propagation was more uniform in MS compared to aggregate with no significant differences in calcium transient duration and transient velocity. Finally, hiPSCs differentiated into contracting tissues in microspheres, with higher CM content and faster contractility than EBs, demonstrating the potential of encapsulation to enhance the consistency, yield, and functionality of cardiac tissue produced in scalable culture systems.

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The HUMIMIC ActSense: A multifunctional device to incorporate electrical functionalities into microphysiological culture systems

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The ability to simultaneously exploit different electrical sensing and actuation techniques in a single device is of paramount importance in the context of multiphysiological systems, as the long-term probing of cells' electrical activity provides fundamental information on dynamic tissue responses and organ-specific reactions.

In this work, we present the HUMIMIC ActSense, a device capable of performing single frequency transepithelial electrical resistance (TEER) measurements, multi-frequency domain impedance spectroscopy analysis, electrical stimulation and multi-channel action potential reading. While simple TEER measurements have been successfully employed to assess the junction dynamics and barrier integrity of endothelial or epithelial models, our electrical impedance spectroscopy technique exploits multi frequency analysis in the 10 Hz-100 kHz range to probe the electrical characteristics of a cultured tissue, thus allowing, for example, the evaluation of the growth, spreading, and differentiation of different cell types at different maturation stages. Moreover, the HUMIMIC ActSense can deliver multiple stimulation profiles, including trains of impulses, square or sine waves, with adjustable amplitude and frequencies up to the kilohertz range. The simultaneous recording of the cells' electrical activity is carried out by 8 differential (16 single ended) sensing sites with a voltage resolution down to microvolts and sampling rate of up to 16,000 samples per second.

These electrical stimulation and sensing techniques are coupled with on-chip integrated multi-electrode array (MEA) technology, featuring conductive poly(3,4-ethylenedioxythiophene): poly(sodium4-styrenesulfonate) (PEDOT:PSS) as the electrode material. This transparent polymer can be easily ink-jet printed on plastic materials at high throughput and allows simultaneous observation via standard optical microscopy techniques, while being characterized by low thermal noise and superior charge injection compared to standard metal electrodes, thus resulting in higher signal-to-noise recordings.

This multifunctional coupling stimulation and sensing functionalities promises to provide researchers with a valuable tool to study the electrical properties of cells cultured in microphysiological systems.

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Modular integration of endothelialized barrier for disease modeling

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The endothelial barrier has apical (tight junction) and basal (adherens junction) junctions and alteration in their organization due to various stimuli results in a leaky or dysfunctional barrier. The remodeling of the barrier is disease-specific, and the consequences depend on the affected organ. A bottleneck in understanding the underlying mechanisms is the lack of an appropriate model to study the barrier. The *ex-vivo* tissue extracts often comprise a mixture of large and small blood vessels which does not recapitulate the leaky regions present in *in vivo* microvasculature. Moreover, the culture conditions may upregulate or downregulate the inflammatory genes and also lack the dynamic flow conditions found *in vivo*. In our study, we have fabricated a modular membrane chip using biologically relevant cells with regulated flow conditions. Our method entails the maturation of the endothelial barrier onto a static transwell-based culture and confirming its establishment through TEER measurement. The transwell membrane is then transferred onto the chip reversibly, and independent flow pressures and flow rates can be maintained on the apical and basal channels using pressure-driven flow pumps. The chip design allows real-time imaging and thus provides insights into cellular interactions and extravasation. We model the blood-brain barrier (BBB) to study Multiple Sclerosis (MS) as the loss of barrier function plays a significant role in the disease progression and pathology. The primary brain microvascular endothelial cells from diseased and healthy mice are utilized to mimic the BBB efficiently. Additionally, we model islet and immune cell interaction for type 1 diabetes (T1D). The genome-wide association studies relating to risk for development T1D suggest a contribution of various gene loci. Here we have iPSC-derived endothelial cells with the risk allele HLA-A2 to interrogate the hypothesis that T1D specific risk alleles result in increased CTL-mediated β-cell killing.

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Mesoscale MPS platform for co-cultures using 3D Hy-MAP (3D hybrid-micromesh assisted patterning)

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Bioprinting has been widely used to fabricate tissue engineering scaffolds and develop *in vitro* tissue/tumor models. However, relatively low resolution of hydrogel structures and long fabrication times due to the extrusion process has limited wider applications. Here, we present a 3D hybrid-micromesh assisted patterning (Hy-MAP) method that combines digital light projection (DLP) 3D-printed micromesh structures that can be used sequential hydrogel patterning. The combination of high resolution DLP-based 3D printing allows selective patterning of different cell types embedded in hydrogels for MPS (microphysiological systems). Various methods, including gel injection, dipping and draining enable construction of mesoscale (1-50 mm) complex 3D hydrogel structures by extending the micropost-based patterning that has been demonstrated in 2D microfluidic channels to 3D channel networks. We established the design rules for Hy-MAP through both analytical and experimental investigations of the capillary bursting pressure (CBP) dependence on the size and geometry of the mesh as well as other physical parameters. To demonstrate the utility of this approach, vascularized tumor microenvironment (TME) was formed using Hy-MAP by culturing endothelial cells, stromal cell mixtures and tumor clusters inside separate but adjacent compartments. Moreover, we evaluated the efficacy of anti-tumor drugs on TME in terms of size and viability of tumors. Combination of two chemotherapeutics, 5-fluorouracil (5-FU) and oxaliplatin, showed suppressed tumor growth and lower viability compared to mono-treatment. The novel approach will provide an alternative method for fabricating mesoscale MPS as well as implantable tissue engineering constructs.

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Human stem cell-based retina on chip – A screening platform for retinal drug development

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Ophthalmologic drug development still largely relies on animal models that are often hampered by translatability issues. Bioengineered human organ/tissue *in vitro* models have the potential to fill this gap. Retinal organoids (ROs) derived from human induced pluripotent stem cells (hiPSCs) contain all known major retinal cell types – including photoreceptor cells, bipolar cells, Müller glia, and ganglion cells – possess an *in vivo*-like retinal layering, and, importantly, build functional synaptic connections. Yet, ROs lack vascularization and cannot recapitulate the important physiological interactions of matured photoreceptors and the retinal pigment epithelium (RPE). We have developed a tailored microfluidic platform, the Retina-on-Chip (RoC), that recapitulates retinal tissue in its full complexity as well as the mimicry of systemic and subretinal drug delivery. This platform integrates hiPSC-ROs embedded in ECM-like hydrogels on top of RPE layers and features a choroidal vasculature-like perfusion. The RoC successfully recreates RPE polarization and the interaction between RPE and photoreceptors resulting in an increased formation of outer segment-like structures, outer segment phagocytosis and calcium dynamics. In collaboration with pharmaceutical partners, the RoC has been applied to study the effect of small molecules on retinal tissue viability and lysosomal activity using fluorescence live imaging. Moreover, the RoC model enabled assessment of AVV gene therapy by *in situ* monitoring of the incorporation of the GFP gene in the retinal tissue, quantification of the infection kinetics as well as characterization of cell tropism. This work demonstrates the potential of hiPSC-based Organ-on-Chip models to promote drug development, provide new insights into the underlying pathology of retinal diseases and serve as the next generation of screening platforms for future gene therapeutic studies.

Presentation: Oral

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Hemorheology and pathophysiology of COVID-19 induced thrombosis predicted by vein-chip

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There is a serious limitation of experimental models that can improve our limited knowledge of the mechanisms that regulate endotheliopathy and venous thrombosis (VT) clinically observed frequently amongst the most severe COVID-19 patients. Also, while observation and study of VT in humans are difficult due to the deep-lying nature of the deep veins in which VT develops, lab animal models do not include the venous valves, which are the sites of unique hemodynamics and thrombus development in humans. We develop a Vein-chip microfluidic platform that includes venous valve architecture, endothelial cells (ECs), and whole blood flow, which can include the three factors of Virchow's triad – endothelial inflammation, stasis of blood flow, and coagulable nature of blood. Our *in silico* and *in vitro* observations with Vein-Chip reveal that incompetent valves and thrombosis changes the blood flow pattern in and around the venous valves. We show that healthy endothelium at the venous valve cusps adapt to the complex flow patterns and have an anti-thrombotic phenotype compared to the venous lumen. But exposure of the lumen to living and replicating SARS-CoV-2 virus and inflammatory cytokines found in COVID-19 patient samples inflames the lumen and the valve endothelium becomes pro-thrombotic. Interestingly, when we directed our investigation to analyze the ACE2 expression on these cells, as ACE2 is the functional receptor of the SARS-CoV-2 virus, we found that ACE2 expression was poor under a static culture, but increased dramatically when venous ECs were exposed to shear stress within the vein-chip. This data supports our hypothesis that ACE2 expression (and therefore, SARS-CoV-2 entry into the endothelium) is dependent on venous hemodynamics and the Vein-Chip model is a highly dissectible platform that will help us to unravel the molecular mechanisms that lead to VT and its treatment strategies for COVID-19 and beyond.

Presentation: Oral

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Integration of Schwann cells to construct a tripartite NMJ model for personalized medicine

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The neuromuscular junction (NMJ) is a specialized synapse between skeletal muscles and motoneurons. The integrity of the NMJ is vital for voluntary muscle contraction, and NMJ dysfunction has been found as an essential pathological mechanism in multiple neurodegenerative diseases such as ALS and Myasthenia Gravis. Another type of cell supporting this motoneuron-muscle connection are non-myelinating Schwann cells which surround the synapse, forming a tripartite structure. An *in vitro* tripartite NMJ model would be superior to a motoneuron-muscle model because Schwann cells are present *in vivo* and help protect, strengthen and insulate the motoneuron-muscle connection. We have previously developed an NMJ model by co-culturing induced pluripotent stem cell (iPSC)-derived skeletal muscle and motoneurons in microfluidic chambers. This study aimed to improve the existing NMJ model through the addition of Schwann cells to better mimic the *in vivo* structure. To achieve this, we validated the medium for the co-culture of Schwann cells and skeletal muscle. The timing of introducing mature Schwann cells into the skeletal muscle culture was optimized to best accommodate the differentiation of myofibers and Schwann cells. With this understanding, mature Schwann cells were then integrated into the muscle chamber of the motoneuron-muscle NMJ model thus achieving an advanced triculture NMJ system featuring a tripartite synapse. Because of the genetic diversity in both familial and sporadic ALS, personalized medicine is highly desired for therapy development. Advanced *in vitro* models are a step towards achieving personalized medicine utilizing iPSC technology. The iPSC-derived tripartite NMJ model would provide a platform for personalized pathogenesis investigation and drug testing.

Presentation: Poster

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Fabrication of a structurally biomimetic *in vitro* cartilage model using textured polydimethylsiloxane and carbon nanotubes

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Creating an *in vitro* platform to support the growth of physiologically relevant articular hyaline cartilage would reduce the need for animal testing to gain an improved understanding of diseases such as osteoarthritis, as well as accelerate the development of therapeutic strategies. With an ageing population, the issue of osteoarthritis is more prevalent than ever and the burden on healthcare is only increasing. In this work, focus was placed on tissue scaffold design, attempting to mimic the physical and mechanical properties of native articular cartilage to enhance the resulting artificial tissue.

Carbon nanotubes (CNTs) were used in this work to simulate the nano-scaled structure offered by proteins naturally found in cartilage, namely collagen. Polydimethylsiloxane (PDMS) was used to mimic the mechanical properties of cartilage. Additionally, PDMS is an excellent material for the formation of textured scaffolds. Introducing macro-scaled, parallel grooves can alter chondrocyte alignment, which varies throughout cartilage zones. It is of utmost importance to be able to control the alignment of cells to create a cartilage construct that truly represents native tissue. Additionally, the proposed PDMS-CNT scaffold allows for mechanical deformation and cellular stimulation.

The application of CNTs to the surface of PDMS-based scaffolds resulted an up to 10-fold increase in cell adherence and 240% increase in proliferation. The textured scaffolds induced the alignment of both chondrocytes and collagen fibers, thus mimicking the structure observed in the superficial layer of cartilage. Chondrocyte phenotype and cartilage formation were analyzed by Raman spectroscopy, and the presence of collagen and the cartilage-specific proteoglycan aggrecan was detected.

This study demonstrates that the introduction of physical features at different length scales allows high level of control over cell behavior and, as a result, tissue formation. Having more reliable early-stage testing will reduce late-stage attrition of drug candidates and therapies.

Presentation: Poster

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Microphysiological heart liver human-on-a-chip system with a skin mimic for evaluating topical drug delivery

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One of the most challenging problems faced by pharmacology and biotechnology companies is the low success rate of compounds that make to regulatory approval. This low percentage of approval is largely attributed to the low accuracy of preclinical predictive *in vitro* and *in vivo* models for drug efficacy and toxicity in humans. Compounds that have been withdrawn from trials were mostly those that cause hepatotoxicity and/or cardiotoxicity. Although most drugs have better bioavailability and consequently are more effective through oral administration, transdermal drug delivery is a successful method for drug administration for prolonged periods of time that can reduce the risk of toxicity. Thus, it is relevant to develop a multi-organ system that efficiently evaluates the toxicity of topically administered drugs to human heart and liver tissues. In this study, a new microphysiological heart-liver (HL) human-on-a-chip system was developed with a skin surrogate for testing drug toxicity associated with transdermal drug delivery. In order to evaluate the system, diclofenac sodium, ketoconazole, acetaminophen and hydrocortisone were administered topically and the toxicity results were compared to those from systematically applying the compounds. The heart-liver system was successful in predicting the effects for both cardiac and liver function changes due to the compounds. The difference in the concentrations of drugs applied topically compared to systemically indicate that the barrier properties of the skin surrogate was efficient. Ultimately, this system can be used for the assessment of potential drug toxicity from dermal absorption as well as to evaluate transport dynamics through the skin in the same system. This new finding could assist in the design of clinical trial protocols and reduce the cost, time, and labor of drug development.

Presentation: Poster

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Detection of cell polarity in renal proximal tubule epithelial tissue by measuring the trans-epithelial electrical resistance in a microphysiological system

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Epithelium is commonly modeled using MPS to evaluate the absorption and excretion of drugs (Bein et al., 2017). Drugs pass through the epithelium by paracellular and/or transcellular pathways. The paracellular pathway is associated with transport in the gap between epithelial cells and is regulated by the barrier function of tight junctions (TJs). The transcellular pathway on the other hand is mediated by membrane transporters asymmetrically located at the apical and basal sides. The emergence and maintenance of cell polarity are essential for the polarized transport of membrane transporters (Harada, 2010).

Cell polarity are therefore crucial indicators to evaluate the absorption and excretion of drugs. The evaluation of cell polarity, however, is limited to methods such as immunocytochemistry of the localized transporter, which is an invasive method and cannot be conducted in real-time.

We applied the TEER measurement technique to non-invasively detect the cell polarity in real-time. The TEER includes the resistance of the other junctions called adherens junctions (AJs) located at the basal side of the TJs. TJs and AJs require extracellular Ca^{2+} to maintain the adhesions, although they are regulated by different adhesion proteins, claudin and cadherin, respectively (Tariq et al., 2015). Therefore, we used the sensitivity of the adhesion proteins to extracellular Ca^{2+} to evaluate cell polarity.

We first designed a bi-channel microfluidic device integrated with a four-probe electrode to measure TEER. It was demonstrated that cell polarity should be considered during the process of Ca^{2+} removal and replacement in explaining the dynamics of TJs and AJs. Our data suggests the TEER measurement can be applied to detect the presence of cell polarity in MPS.

Presentation: Poster

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Engineered immunocompetent intestinal models: Applications in cancer immunotherapy and beyond

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The high late-stage attrition of cancer immunotherapies can be attributed in large part to the poor correlation of drug pharmacodynamics between preclinical models and patients. Organoids are powerful mimetics of human parenchymal tissue, offering previously unparalleled opportunities for modeling organ development, function and disease. However, they are largely devoid of immune and stromal compartments, which are indispensable for capturing the effects of immunomodulatory drugs. We have rendered intestinal organoids competent in simulating immune responses by incorporating salient aspects of peripheral and tissue-resident immunity. We have validated the systems as platforms for routine safety profiling of T-cell bispecific antibodies, finding that they can recapitulate clinical adverse events previously overlooked by conventional preclinical models.

Furthermore, we have developed approaches leveraging geometrically-guided organoid formation to build next-generation engineered intestinal models that are long-lived, exhibit superior maturation and feature *in vivo*-relevant microanatomy and architecture. These systems afford the modularity and compartmentalization required to capture the complex functional interactions between diverse cells types that underlie not only drug-induced pathologies but also disease. As such, beyond predicting clinical adverse events, they open previously inaccessible avenues into modeling, understanding and treating complex conditions, including cancer and inflammatory bowel disease.

Presentation: Oral

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Next generation organ-chips made of blood: Mimicking patient-specific vascular pathophysiology in a vessel-chip composed of blood-derived endothelial cells

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Advances in tissue engineering and organ-chip technology have accelerated *in vitro* research of the vascular system. Incorporating patient-derived endothelial cells have been suggested to advance organ-chips in positively impacting preclinical modeling of precision medicine. Here, we report the use of Blood Outgrowth Endothelial Cells (BOECs) directly isolated from patient blood via simple phlebotomy as an alternative to the significantly difficult to obtain primary and iPSC-derived endothelial cells for organ-chip applications. BOECs exhibit the gold-standard endothelial hallmarks of the "cobblestone" morphology *in vitro*. When compared to primary and iPSC-derived endothelial cells, the blood-derived ECs reveal similar levels of growth rates, migration capabilities, *de novo* vessel formation, response to fluid shear stress and exogenous cytokine inflammation *in vitro*. To validate patient-specificity, we derive BOECs from diabetic and sickle cell disease (SCD) patients with known differences in clinical severity of the disease. Through next generation RNA sequencing and proteomics of these cells, we reveal that patient-derived BOECs are activated and clinically-known more severe patients exhibit relatively higher endotheliopathy than the mild cases. This is further confirmed by through endothelialized vessel-chips with BOECs which reveal differential platelet and immune cell adhesion, and thromboinflammation between severity of patients. In conclusion, organ-chips constituting blood-derived endothelial cells have strong potential to be employed in preclinical research for developing more robust and personalized next-generation disease models that will ultimately enable clinicians in identifying individuals at high risk of vascular complications or stroke.

Presentation: Poster

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Tumor microenvironment-chip: Discovery of platelet immunopathology & combinatorial therapy in cancer

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Platelets extravasate from the circulation into tumor microenvironment, enable metastasis and confer resistance to chemotherapy in several cancers. This platelet pathophysiology is suggested to be regulated by the vascular endothelium and its immune function. Therefore, arresting tumor-vascular and tumor-platelet crosstalk with effective and atoxic, antivascular and antiplatelet agents in combination with anticancer drugs may serve as an effective cancer treatment strategy. To test this concept, we created a new tumor microenvironment-chip (or Tumor-Chip) which recapitulates platelet extravasation through the endothelium and its consequences. By including gene-edited tumors and RNAseq on-chip, this organ-chip revealed temporal dynamics of vascular disintegration due to ovarian cancer cells, differential increase in inflammatory biomarkers and alteration of a number of regulatory genes. We also found that platelets and cancer cells interact through glycoprotein GPVI and tumor galectin-3 molecules. Finally, as proof-of-principle of a clinical trial, we revealed that atorvastatin therapy and a novel GPVI inhibitor, Revacept, impairs metastatic potential of cancer and even improves chemotherapy. Since these drugs do not impair hemostasis, our work shows that they are a safe cancer therapeutic. Our data are validated by observations in patient tumor samples, and therefore, we propose that our Tumor-Chip is a new platform to model the cancer-vascular-hematology nexus and analyses of potential therapeutics.

Presentation: Poster

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Development of a multi-organ opiate overdose and recovery platform by differentiation of preBötC neurons and nociceptors from iPSCs

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Opioid overdose is the leading cause of these drug's lethality, posing an urgent need for investigation. The key brain region for inspiratory rhythm regulation and opioid-induced respiratory depression (OIRD) is the preBötzinger Complex (preBötC) and current knowledge has mainly been obtained from animal systems. This study aimed to establish a human-based opiate overdose model using the PreBötC neurons or nociceptors derived from human induced pluripotent stem cells (hiPSCs). A *de novo* protocol was developed for differentiating preBötC neurons from hiPSCs. These neurons express essential preBötC markers analyzed by immunocytochemistry and demonstrate expected electrophysiological responses to the excitatory neurotransmitter glutamate as well as multiple preBötC modulators (ATP, substance P, somatostatin) as analyzed by patch clamp electrophysiology. Furthermore, the dose-dependent inhibition of these neurons' activity was demonstrated for four different opioids (DAMGO, fentanyl, methadone, codeine), with identified IC50's comparable to the literature. Moreover, inhibition by all the four tested opioids was rescued by naloxone in a concentration dependent manner. As an alternative, an overdose model utilizing iPSC-nociceptors was also developed demonstrating a dose-dependent excitation by ATP and its subsequent silencing by DAMGO. To investigate the opioid drug efficacy and off-target toxicity, the iPSC-preBötC model was then adapted onto microelectrode arrays (MEA) and integrated into a 5-organ microfluidic system containing preBötC neurons, hepatocyte, cardiomyocytes, skeletal muscle and kidney renal proximal tubule cells (RPTCs), in which the opioid overdose effect and its recovery by naloxone was reproduced. The iPSC-preBötC model is a crucial step for investigating OIRD and combating the overdose crisis. Integration of this overdose model into the multi-organ system provides a powerful platform for evaluating drug efficacy and toxicity for therapy development.

Presentation: Poster

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Characterization of novel human brain organoids to study cellular resilience to toxicants using targeted cell ablation

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Current *in vitro* models are powerful tools to study the molecular pathways involved in neurotoxicity but many *in vitro* toxicity tests have only studied the acute effects of compounds at high concentrations which does not reflect real-life exposures which are usually repetitive (Harris et al., 2018). The cells' ability to cope with environmental changes such as repetitive toxicant exposure can be described as Cellular Resilience (Smirnova et al., 2015). Therefore, there is a need to develop and characterize a complex model to study cellular resilience and neurotoxicology in the context of neurodegeneration (e.g., Alzheimer's and Parkinson's diseases). Previously, we showed cellular resilience in our 3D dopaminergic neuronal model after rotenone exposure (Harris et al., 2018). However, this model consists of one cell type-dopaminergic neurons. Therefore, here we aim to characterize a novel human brain organoid model as a more complex and *in vivo* like model to study cellular resilience. To research this, we modified our lab's hiPSC-derived brain organoid model using CRISPR/Cas9 to insert nitroreductase (NTR) attached to a moxVenus fluorescent reporter under neuronal NeuroD6 and dopaminergic neuron-specific DAT gene promoters. Expression of NTR allows these organoids to be an inducible cellular ablation model, as NTR can be activated upon the use of a prodrug to ablate cells expressing either the NeuroD6 or DAT gene. We differentiated these genetically modified iPSCs into brain organoids and let them mature over eight weeks. Immunohistochemistry and RT-PCR were performed to characterize organoid differentiation and cellular composition. Rotenone is used in our lab as a model PD-inducing toxicant. Expression and activation of NTR can be used as a positive control for PD to compare the acute response of the reporter organoids to rotenone treatment, followed by recovery, and eventually resilience. This will allow us to study how DAT+ and NeuroD6+ neurons are affected by rotenone and whether they are resilient to a second exposure. Moreover, this inducible cell ablation organoid model has potential to serve as a high-throughput screening pipeline for drugs protective against neurodegeneration.

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Presentation: Poster

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Chemotherapy-induced peripheral neuropathy using a nerve-on-a-chip microphysiological system

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Organs-on-Chip devices mimicking *in vivo* physiology have potential to identify effects of chemical and drug exposure in preclinical stages of drug development without relying heavily on animal models. Toxicity is one of the leading reasons that drugs are removed from the market with neurotoxicity responsible for 16% of all withdrawals. Peripheral nerves are particularly susceptible to toxic off-target effects that can result in permanent sensory and motor deficits.

We designed a biomimetic rat nerve-on-a-chip (RNoaC) construct that promotes axon growth analogous to mature nerve anatomy and is the first 3D *in vitro* nerve model to collect electrophysiological and histomorphological metrics, the gold standard methods used to assess *in vivo* neuropathophysiology.

Here we culture embryonic rat dorsal root ganglia in a hydrogel construct to demonstrate its potential to screen for implications of nerve dysfunction in chemotherapy-induced peripheral neuropathy. Mature RNoaC constructs after 28 days *in vitro* were exposed to the chemotherapeutics bortezomib, oxaliplatin, paclitaxel, or vincristine for 7 days. Then, axons were electrically stimulated to elicit compound action potential from each construct, used to assess nerve conduction velocity (NCV) and peak amplitude (AMP), which are clinically analogous metrics indicative of peripheral neuropathies. Histological analysis and cell viability assays were also performed at this time point to observe underlying changes in the tissue after drug exposure.

All chemotherapeutics decreased NCV and AMP in a concentration-dependent manner. At high drug concentrations, NCV and AMP were 10-60% lower than vehicle control. Histopathological analysis revealed hallmarks of peripheral neuropathy, including decreases in myelinated fiber density and increases in the percentage of degenerated fibers. Calculated IC50 values indicate that significant decreases in electrical function occurred before a decrease in viability.

Our data suggest that electrophysiology recordings collected from our RNoaC platform can closely track subtle pathological changes in nerve function, with distinction between electrical functional deficits and general cytotoxicity. Our RNoaC platform

is unique in that it provides characteristic output metrics of both *in vitro* and *in vivo* studies. The ability to collect clinically relevant data from RNoaC suggests it can be an effective tool for *in vitro* preclinical screening of peripheral neuropathy.

Presentation: Oral

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Human tissue models supporting clinical development and personalized medicine

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In the era of precision medicine, drug discovery and development face unprecedented opportunities for product and business model innovation, fundamentally changing the traditional approach of how drugs are discovered, developed and marketed. Critical to this transformation is the adoption of new technologies in the drug development process, catalyzing the transition from serendipity-driven to data-driven medicine. Key components of Precision Medicine are multi-omics profiling, digital biomarkers, model-based data integration, artificial intelligence, biomarker-guided trial designs and patient-centric companion diagnostics.

While microphysiological systems (MPS) successfully have made their way into early drug discovery stages for disease modeling, target identification and toxicity assessment, few examples exist for application of human tissue models supporting Precision Medicine during clinical stages. We aim at exploring application of MPS to optimize the safe and effective use of medicines at the individual patient level or patient-subgroup level. To do so, building on patient-specific data is key, e.g., biomarkers and genetic profiling, that allow the generation of respective MPS models displaying these characteristics. Furthermore, we are exploring paths that involve making direct use of patient-derived tissue for generating human tissue cultures from individuals enrolled in trials. Such approaches require automation and scalability for the generation of organoids that are suitable to timelines and the decentralized set up of typical clinical trials. In a first phase, one could envision using MPS in co-clinical trials to compare drug responses in organoids to clinical responses in the corresponding patient. Next to predicting patient response at the individual level, MPS could support mechanistic understanding of observed effects, defining optimal treatments for specific patient populations, selecting optimal drug combinations and optimizing overall trial design by prior *in vitro* testing of the patient group in scope.

If successful, such new avenues could transform treatment choices in the clinic to guide precision therapy for patients, improving patient experience by selecting patients that will benefit most and develop tailored approaches to manage toxicities which would positively affect patient burden & healthcare costs leading to tangible and measurable patient outcomes.

Presentation: Oral

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Reproducibility in cell culture: Replacing fetal bovine serum

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Reproducibility is key for all scientific developments and industrial production. This holds true especially in cell culture, where the smallest impurities could cause false conclusions or process malfunctions. Nonetheless, it is astonishing that one of the most ubiquitous cell culture supplements is also known to be a cause for irreproducibility: Fetal bovine/calf serum (FBS/FCS); used due to its promoting effect on cell growth and proliferation. Its undefined nature combined with high batch-to-batch variability and risk of contaminations place it as an additional uncertainty factor introduced in cell culture.

Here we present examples for such uncertainties and potential risks arising from the use of FBS (van der Valk et al., 2018). These can be overcome by the use of replacements which paves the way towards reliable, reproducible and safe science (Weber et al., in press). Furthermore, due to its unsolved animal welfare issues, replacing FBS will also contribute towards cruelty-free research. Therefore, we demonstrate two cell culture supplements: Human platelet lysate (hPL) and a do-it-yourself chemically defined medium.

When derived from expired blood donations, hPL can be an ethically acceptable and xeno-free replacement for animal-derived FBS, especially suitable in tissue engineering, organoids and cell therapy. As a natural product, hPL can still be a source of variability, thus for certain applications a switch to chemically defined media would be the solution. We will provide an example for L929 and Caco-2 cell lines and present the publicly available "Fetal Calf Serum-Free Database" (https://fcs-free.org/), hosted by the 3Rs-Centre at Utrecht University.

FBS-free media do not (yet) exist for all cell types, so these (or a single universal medium) have to be developed (van der Valk, 2022). Furthermore, all animal-derived materials should be completely replaced or otherwise eliminated. Transition to an animal-component-free cell culture is not only an ethical but a scientific necessity for modern science, health and production.

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Presentation: Poster

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Systems engineering of microphysiometry

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The international council on systems engineering defines systems engineering as "*Systems Engineering is a transdisciplinary and integrative approach to enable the successful realization, use, and retirement of engineered systems, using systems principles and concepts, and scientific, technological, and management methods*". Microphysiometry is the *in vitro* measurement of the functions and activities of life or of living matter (as organs, tissues, or cells) and of the physical and chemical phenomena involved on a very small (micrometer) scale (Wiest 2022; Brischwein and Wiest 2018). Microphysiometry emerged in the last quarter of the 19th century and laid grounds for the success of today's microphysiological systems or organ-on-a-chip technologies. However, the field of biomedical sciences is still in the reproducibility crisis. To guarantee reliable results, it is essential to maintain a quality management system and to eliminate human errors by automation (Pamies et al., 2022). Here, aspects of the experimental conditions such as climate control, fluidic systems and the use of chemically defined cell culture media are discussed and recommendations how to improve reproducibility of cell-based assays are given. Finally, a microphysiometric assay which allows the investigation of recovery effects will be presented and an outlook toward an automated system is presented.

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Presentation: Oral

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The cellasys #8 assay: An automated and standardized assay to address recovery effects

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Cell culture media (CCM) is a critical factor which profoundly influences cells, the observed biology and, most importantly, the research results. However, current metabolic and morphological assays still rely on manual, time-consuming, and destructive methods, which ultimately slow down the development of promising CCM formulations. In addition, endpoint measurements do not only increase the number of required samples, but also lack the ability to provide real-time data and, hence, lack information about recovery effects. To address these challenges, we have established an automated and standardized microphysiometry method to allow non-invasive, label-free and real-time monitoring of metabolic and morphological parameters for the evaluation of novel CCM formulations. In particular, miniaturized pH, oxygen and impedance microsensors allow measurements to read out changes in cell adherence and vitality in real time during an automated and integrated medium change (Wiest, 2022). This complex structure thus allows the evaluation of two crucial features: On the one hand, the treatment with a novel CCM is investigated to identify the applicability. On the other hand, the recovery phase is evaluated when the assay switches back to the reference medium (e.g., established CCM). To demonstrate the applicability of the cellasys #8 assay, we evaluated our established serum-free CCM formulation (DME/F12+ITS) for L929 cells, which are generally cultured in DMEM supplemented with fetal bovine serum. The assay indicated no changes during the treatment as well the recovery phase, demonstrating that the serum-free CCM is able to replace the serum-based CCM without changing cell adherence and vitality. In combination with our previously established organ-on-chip systems (e.g., skin (Schmidt et al., 2020), liver (Alexander et al., 2018)), the platform is able to accelerate the development of novel CCM for microphysiological systems by decreasing time and effort and, hence, bring more reproducible and physiologically relevant system to industry in the future.

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Presentation: Poster

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Robust strategies for generating perfusable microvasculature-on-a-chip models for cancer studies

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Microvascular network (MVN) is an indispensable element in microphysiological systems. Numerous studies have established diverse MVN models, among which microfluidic devices are widely used for seeding primary vascular cells, e.g., human umbilical vein endothelial cells (HUVECs), dermal ECs, brain ECs, human lung fibroblasts (FBs), and pericytes. However, primary cells derived from different donors have genotype diversities and batch-to-batch variations. And these primary cells have limited proliferation capacity when cultured *in vitro*. All these limitations hamper the reproducibility of MVN formation. In addition, consistently generating perfusable MVNs of physiological morphology and dimension has proven to be challenging.

To overcome the limitations of using primary cells, we took advantage of the immortalization technique (exogenously expressing human telomerase reverse transcriptase, hTERT) to establish stable cell sources for MVN formation. These immortalized vascular cells can form functional MVNs even over 20 passages. To generate perfusable MVNs with physiological diameter, we investigated how initial seeding parameters determine key characteristics of MVN formation, utilizing immortalized vascular cells we developed. Furthermore, we established a novel 2-step seeding strategy that forms large open lumens at the outer layer region connecting capillary-like MVN in the central region. In this way, the capillary-like MVNs are fully perfusable. 2-step strategy is amenable to many types of endothelial cells, stromal cells, and extracellular matrices, including those derived from patients and iPSC-derived vascular cells. MVNs made with immortalized vascular cells by 2-step seeding strategy can be used to study tumor cell extravasation, circulating tumor cluster trapping, immune cell trafficking, and tumoral organoid vascularization.

In summary, we developed immortalized vascular cells and a novel seeding strategy for MVN formation in microfluidic devices for cancer studies.

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Kidney epithelial cells are active mechano-biological fluid pumps

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Using a novel microfluidic platform to recapitulate fluid transport activity of kidney cells, we report that renal epithelial cells can actively generate hydraulic pressure gradients across the epithelium. The fluidic flux declines with increasing hydraulic pressure until a stall pressure, at which the flux vanishes – in a manner similar to mechanical fluidic pumps. The developed pressure gradient translates to a force of 50-100 nanoNewtons per cell. For normal human kidney cells, the fluidic flux is from apical to basal, and the pressure is higher on the basal side. For human Autosomal Dominant Polycystic Kidney Disease cells, the fluidic flux is reversed from basal to apical with a higher stall pressure. Molecular studies and proteomic analysis reveal that renal epithelial cells are sensitive to hydraulic pressure gradients, developing different expression profiles and spatial arrangements of ion exchangers and the cytoskeleton in different pressure conditions. These results, together with data from osmotic and pharmacological perturbations of fluidic pumping, implicate mechanical force and hydraulic pressure as important variables during morphological changes in epithelial tubules, and provide further insights into pathophysiological mechanisms underlying the development and transduction of hydraulic pressure gradients.

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Establishing a high-throughput viability assay in colorectal cancer (CRC) organoids for long-term drug treatment

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Background: Colorectal cancer (CRC) is the second most deadly cancer and the third most prevalent malignancy globally. The 5-year survival rate for CRC and metastatic-CRC remains at 64% and 12% respectively, suggesting a need for urgent effort towards developing better therapies. Patient derived organoids (PDOs) are three dimensional complex structures derived from adult stem cells. PDOs faithfully recapitulate histological, molecular, and genetic characteristics of the parental tissue and thus serve as an excellent model for developing clinically relevant treatments. Here we report the development of a long-term 384-well plate high-throughput assay using CRC PDOs for drug screening applications.

Materials and methods: CRC organoid lines (6) were expanded and processed for histopathology and OMICS (bulk RNA-seq, WES, and DNA methylation array) analysis. Assay development was performed in 384-well plate under suspension culture to optimize organoid seeding size and density for growth over 10 days. Assessment of organoid viability was used as the primary endpoint. Assay performance was validated by testing response to CRC standard of care compound SN-38. Data analysis and statistics were performed on GraphPad Prism.

Results: All 6 CRC organoid lines were successfully expanded and biobanked. Histopathology confirmed epithelial nature and ruled out the possibility of fibroblast contamination/EMT (Pan-CK+ and Vimentin-) in organoid culture. Furthermore, strong Ki-67 staining corroborated robust proliferative nature of the tumor organoids. PDOs demonstrated continuous growth for 10 days in 384-well plates under suspension culture without media change. A starting size of 20-40 microns and seeding density of 200/400 organoids/well were identified as robust assay conditions ($Z' > 0.4$ and $S:B > 250$) for 8-day viability screen. PDOs responded robustly to SN-38 (IC50: ~0.5 nM) and velcade (IC50: ~0.03 nM) and demonstrated strong agreement between plates.

Conclusions and future directions: PDOs are amenable for establishing robust high-throughput assays for long-term drug treatment. Ongoing work involves: 1) Genomic, transcriptomic and epigenomic characterization of PDOs, and 2) Drug screens.

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Evaluation of pegcetacoplan in ameliorating ALS disease phenotypes in human-on-a-chip neuromuscular junction system

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Neuroinflammation is considered a pathological feature of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). The complement system is a key mediator of neuroinflammation and is known to drive neurodegenerative mechanisms in ALS. For example, complement (e.g., C3 and C5) activation products are elevated in ALS patients and may trigger an inflammatory response leading to motoneuron death, NMJ denervation and heightened complement expression. Inhibition of complement C3 activation with pegcetacoplan could preserve the neuromuscular junction (NMJ) and unlock a possible therapeutic pathway for ALS. However, preclinical models to evaluate the effect of the complement system and potential therapeutic interventions on NMJ function are necessary.

To assess the impact of complement sera and pegcetacoplan on neuroinflammation and NMJ function, we adapted an established *in vitro* bipartite human-on-a-chip NMJ platform by adding Schwann cells, microglia, and monocytes/macrophages. This novel quinta-partite NMJ platform is composed of WT or ALS diseased (superoxide dismutase (SOD1) (E100G) or TAR DNA binding protein (TDP-43)) skeletal muscle, Schwann cells, and wild-type (WT) immune cells – microglia and monocytes/macrophages separated by microtunnels from WT or ALS diseased motoneurons and WT microglia. These systems are cultured with and without complement sera and pegcetacoplan and functionally stimulated and evaluated for NMJ number, fidelity, and complement deposition.

This study aims to describe the potential applications of a human-on-a-chip model system designed to evaluate the role of C3 inhibition and may help generate the earliest evidence that modulating C3 has a role in ALS disease progression.

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iPSC-derived microglia incorporation into human BrainSpheres for prediction of seizure-inducing properties of toxic chemicals

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There is an urgent need for systems to accurately predict neurotoxicological properties of potential threat agents that may produce seizures, both for the armed forces as well as the pharmaceutical industry. Animal models have been the primary platform for chemical and toxic threat determination so far. However, new therapeutics are often not tested for seizurigenic potential until the clinical phase of drug development, at which point several millions of dollars and years of work can be rendered meaningless if the compound is seizurigenic. *In vitro* solutions to this problem are necessary, however, current cell-based models are limited by cell type, interspecies differences, the expression of physiologically relevant receptors and lacking the complexity needed for a systemic endpoint. In order to accurately predict seizurigenicity using an *in vitro* system composed of human cells that incorporates multiple cell types and possesses the electrophysiological properties of the human brain required to assess seizurigenicity is needed. Human iPSCs (hiPSCs) are emerging as powerful tools for human neurotoxicity testing and prediction. In 2017, Pamies et al. at Johns Hopkins University, developed an iPSC-based brain organotypic model. These brain organoids can be used for neurotoxicity testing as well as being well suited for determining possible seizurigenic properties of chemical agents, as they are spontaneously electrical active. Brain organoids are composed of neurons, astrocytes and oligodendrocytes, however they notably lack microglia, the tissue resident macrophages of the brain. Microglia play an important role in maintaining synaptic function and are important in post-seizure remodeling. Our approach to integration was informed by embryonic development, wherein macrophage progenitors (MPs) seed the putative brain. To model this, we differentiated iPSCs into MPs, then used these cells in combination with neural progenitor cells to produce a microglia-containing organotypic model. This works adds to cerebral organoids ever increasing recapitulation of the human brain.

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Advanced imaging techniques to improve neurological disease modeling in hiPSC derived cerebral organoids

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Imaging induced pluripotent stem cell-derived cerebral organoids is not a trivial process due to their thickness and density, which significantly limit their application for basic neuroscience analyses. While the collection of methodologies for organoid imaging is growing, each must be considered and optimized for specific types of organoids. Multifactorial approaches to choosing reagents, sample preparation, and imaging modalities are required to overcome current challenges of consistently and easily obtaining high-quality, standardized images of 3D organoids. There are two main impediments to imaging organoids due to their inherent structural properties. First is light scattering, which limits the transmission of light. In order to address this, we have adapted and optimized tissue clearing protocols to be used with cerebral organoids. The second significant obstacle is antibody/probe penetration. Thus, we have developed a protocol for fractionating commercially available antibodies to create labeled F(ab') domains. This new probe is roughly 1/3 the size of an IgG molecule and should facilitate more complete penetration into intact organoids. Finally, we have employed expansion microscopy, wherein the effective resolution one is able to obtain is increased through isotropic swelling of a sample. This is achieved with the *in situ* creation of a polymer gel to which proteins in a sample are covalently bound, the resulting tissue is less dense, more permeable and optically transmissive. By combining our previously tested optimizations, we plan to utilize these organoids to create an exact 3D replicate using light sheet microscopy for image analyses that have otherwise been unattainable. The structural renderings are processed using software capable of inter alia dendrite tracing, Sholl analysis, spine morphology and density assessment, and synaptic colocalization, enhancing understanding of structural and functional alterations in the disease model. These advancements to imaging organoids improve their ability to recapitulate the complexities of the human brain, producing clinically-relevant results.

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The BioSystics analytics platform: A comprehensive analytical platform creating actionable knowledge from advanced 3D experimental model, animal, and human data to improve human health and safety

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Increased adoption of advanced 3D experimental model systems for basic biomedical research, drug discovery, drug development and preclinical trials has resulted in a rapid proliferation of experimental models. Selection and implementation of the optimal model, requires a single, powerful analytical platform to efficiently turn experimental data into reproducible, actionable information and knowledge. The BioSystics mission is to transform the rapidly evolving field of *in vitro* experimental models of disease and ADME/Tox ranging from traditional cell-based assays to spheroids, to organoid microphysiological systems (MPS) and structured MPS through a comprehensive computational and systems biology analytics platform. The BioSystics Analytics platform (BioSystics-AP), formerly the MPS-Db, captures, manages, analyzes, shares and computationally models complex data sets from *in vitro* experimental models, animal models and human clinical data creating actionable knowledge and predicting biological outcomes that will accelerate and optimize basic biomedical research, drug discovery/development, preclinical trials, as well as cosmetic, industrial and environmental chemical testing. The BioSystics-AP links to external databases to provide information on drugs, assays, preclinical and clinical data for model design, study design and to develop computational models. It provides an organized workflow platform for implementing MPS studies, capturing data in a central location for easy analyses, and incorporates statistical analyses to assess reproducibility of experimental models, comparison of performance across MPS models, and assay power. Computational modeling of multiscale data permits a better understanding of mechanisms of disease progression and compound ADME-TOX, as well as exploration of therapeutic strategies. As a centralized resource, the BioSystics-AP facilitates sharing data within a lab, with collaborators, or with the research community. There are two versions of the platform: one for non-profits, and a commercial version for installation and integration with corporate infrastructure for for-profit organizations, which is in beta-testing.

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Interstitial flow promotes the formation of functional microvascular networks *in vitro* through upregulation of matrix metalloproteinase-2

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Self-organized microvascular networks (MVN) have become key to the development of many microphysiological models and a valuable tool for diverse applications in tissue engineering. However, the self-organizing nature of this process combined with variations between types or batches of endothelial cells (ECs) in their ability to form functional vessels often lead to inconsistency or failure to form a functional MVN. Since interstitial flow (IF) has been reported to play a beneficial role in angiogenesis, vasculogenesis and 3D capillary morphogenesis, we systematically investigated the role IF plays during neovessel formation in a customized single channel microfluidic chip for which IF has been fully characterized. MVN formed under IF exhibited improved morphologies compared with MVN grown under static conditions with higher percent coverage area (59.3 ± 4% vs. 44.5 ± 8.3%), larger vessel diameters (69.9 ± 2.3 μm vs 44.3 ± 5.4 μm) and greater network perfusability (95.3 ± 2.8% vs. 59.4 ± 38.1%). Through a series of inhibitory experiments, we further demonstrated that IF treatment improves vasculogenesis through upregulation of matrix metalloproteinase-2 (MMP-2). Following this mechanism, we successfully implemented a novel strategy involving the interplay between IF and MMP-2 inhibitor to regulate key morphological parameters of the self-organized MVN, with perfusability well maintained. The revealed mechanism and proposed methodology were further validated with brain specific MVN developed from triculture of primary human brain ECs, pericytes, and astrocytes. Our findings and methods have the potential to be widely utilized to boost the development of various organotypic MVN and could be incorporated into related bioengineering applications where perfusable vasculature is desired.

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Dissecting the role of cGAS-STING pathway in SARS-CoV-2 induced endotheliitis using a lung-on-chip model

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Severe manifestations of COVID-19 result in microvascular thrombosis in the lung and a systemic endotheliitis, but the direct role of the vasculature in the pathology remains unclear. This is in part because endothelial cell monoculture models are resistant to direct infection with SARS-CoV-2 and do not show signs of inflammation. We used a vascularized lung-on-chip model populated with primary human alveolar epithelial cells, monocyte-derived macrophages, and lung microvascular endothelial cells to study the early dynamics of infection in the alveolar space – itself the site of later stages of clinical infection. In our initial report (Thacker et al., 2021), we showed that SARS-CoV-2 RNA and proteins can be rapidly detected in endothelial cells secondary to epithelial cell infection, although endothelial infection is not productive. Infection on-chip leads to a progressive loss of CD31 expression and endothelial barrier integrity, a pro-coagulatory microenvironment, and both NF-kB and type I interferon (IFN) inflammatory responses. These observations are in good agreement with clinical reports of endothelial inflammation in COVID-19.

Building on this foundation, I will describe more recent work (Di Domizio et al., 2022) that links the endotheliitis observed on-chip with endothelial cell activation skin lesions and lung tissue samples from patients with moderate to severe COVID-19. Working as an interdisciplinary team of immunologists, bioengineers, and clinicians, we leveraged the lung-on-chip model to provide insights into the underlying disease mechanisms. We found that SARS-CoV-2 infection leads to altered mitochondrial function in endothelial cells, which activates cGAS-STING signaling through mitochondrial DNA release. This leads to cell death and type I IFN production. Together with evidence from both *in vitro* and *in vivo* studies, we thus demonstrate that inhibition of cGAS-STING pathway is a highly promising host-directed therapeutic approach for COVID-19.

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Presentation: Oral

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Efficacy of doxorubicin against pediatric brain stem glioma at intracerebral-like concentrations in a cancer-on-chip system

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WHO grade IV H3K27M-altered Diffuse Midline Glioma (DMG/DIPG) is a highly malignant brain cancer with an abysmal prognosis. Less than 5% of patients are alive 2 year after diagnosis. Efficacy of chemotherapy is hampered by the blood brain barrier (BBB). With the advent of methods to temporarily open the BBB such as Focused Ultrasound-mediated BBB disruption (FUS-BBBD), previously ineffective oncology drugs could be effective. Animal models of DMG however, are technically demanding and not suitable for screening and repurposing hundreds of available marketed oncology drugs. *In vitro* drug screening methods, on the other hand, often apply time-constant drug concentrations without taking human pharmacokinetics and the blood-brain barrier into account.

Here, we have developed a cancer-on-chip system for evaluating the efficacy of drugs at dynamic, intracerebrally achievable concentrations against DMG. Dynamic drug concentrations are administered by programmed syringe pumps. The chip consists of a straight channel for supplying the dynamic drug concentrations to wells with a diameter of 2 millimeter, in which DMG cells are grown. Doxorubicin was selected as a pilot drug as drug concentrations needed *in vitro* to reduce growth by 50% (IC50) are within achievable plasma concentration in patients, and doxorubicin was previously shown to be subject to enhanced to-brain delivery by FUS-BBBD. GFP-transfected DMG cells were cultured in Matrigel on-chip, to mimic diffuse growth in a solid environment and fluorescence was used as a readout of proliferation. Free doxorubicin concentration in the brain extracellular fluid (bECF) was estimated at ~10% of plasma values, based on the brain-to-muscle ratio method. After application of this hypothesized bECF pharmokinetic (PK) profile, no growth inhibition on-chip was observed, in contrast to on-chip growth inhibition by 48 hours exposure to 1 µM doxorubicin. The approach used here might better predict (the absence of) treatment effects in patients, by dynamically mimicking real-life plasma and brain PK profiles. Future research should elucidate whether assay representativeness can be increased by changing Matrigel to a more brain-like extracellular matrix, and whether this setup can aid in the discovery of other drug (combinations) that can be effective in patients, with and without FUS-BBBD.

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Introducing dynamic mechano-modulation in brain-on-chips

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In this work, we hypothesized that a combination of a hydrogel with a relatively low Young's modulus and a nanoscale membrane actuator forms an interface to distribute forces of a mechanical stimulus such that this mimics a neural extra-cellular matrix (ECM). We preliminary explored such an interface for its bio-functions by casting a selected hydrogel of photo-polymerizable gelatin methacryloyl (GelMA) atop of SH-SY5Y cells seeded on a microscope glass slide. Cells were hence sandwiched between a material of very high Young's modulus (glass) and a material being far softer (hydrogel). By wide-field white light microscopy, fixed cells formed an elongated, branched, and spreading morphology after day 5, which is typical for neurons. Additionally, fluorescent z-stack confocal microscopy images confirmed cells displaced from the stiff surface up to 60 µm into the soft hydrogel matrix.

We propose to use the GelMA layer as an advanced ECM mimicry in conjunction with a nanoscale membrane actuator, which is a specific BoC design previously developed in our group. This BoC is based on nine independent controlled microfluidic airflow channels that can each locally actuate a polydimethylsiloxane (PDMS) membrane and are evenly distributed across the culture reservoir's diameter. Former studies on SH-SY5Y cultured in the same BoC but in a 2D culture format already revealed that modulating exerted forces over time matured a neural network faster. When a hydrogel is placed on top of the deforming membrane, it can elicit a mechanical force field in all three of the dimensions of the hydrogel. Consequently, a biochemical signaling cascade will follow upon actuation among the cells distributed in this advanced three-dimensional (3D) material system. This way of interrogation yields eventually a distinguished 3D organization of cells upon maturation of the culture dependent on the specifics of the exerted force field vectors.

In conclusion, we progressed on the design of a mechano-modulating BoC, which will allow us next to study enriched neuronal functions *in vitro*. In future experiments, we will replace the SH-SY5Y neuroblastoma cell line with stem cell-derived neurons in our animal-free BoC.

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Organ-on-chip models recapitulating complex human immunocompetent tissues

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Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem cells (hiPSC) has led to the emergence of a new paradigm of drug development using human patient- and disease-specific tissue models. One promising approach to generate these models is the integration of µ-tissues derived from hiPSCs or biopsies into microfluidic devices tailored to create microphysiological environments and recapitulate organ-level functionality. Such Organ-on-Chip (OoC) models combine human genetic background, *in vivo*-like tissue structure, physiological functionality, and "vasculature-like" perfusion. The mimicry of aspects of the human immune system with OoCs is a multi-faceted challenge and a major opportunity at the same time: The complexity of the immune system, significant species-differences in immune response, and large population heterogeneities have created an immense need for human-relevant immunocompetent models; especially since the immune systems is a key contributor to many of the most prevalent diseases nowadays and immunomodulatory aspects play a major role for both efficacy and safety of modern therapeutical approaches.

Using microfabrication techniques, we have developed a variety of OoCs that incorporate complex human 3D-tissues and keep them viable and functional over multiple weeks, including Tumor-, Retina-, Choroid-, Heart, Pancreas- and Adipose-on-chip" models. The OoCs generally consist of three functional components: organ-specific tissue chambers mimicking *in vivo* structure and microenvironment of the respective tissues; "vasculature-like" media channels enabling a precise and computationally predictable delivery of soluble compounds (nutrients, drugs, hormones); "endothelial-like" barriers protecting the tissues from shear forces while allowing diffusive transport. To recapitulate human immune responses, we successfully integrated components of the innate and adaptive immune system (tissue-resident and circulating peripheral immune cell) into the OoCs and demonstrated the complex interplay of different cell populations as well as their importance for pharmaceutical safety and efficacy assessment.

In general, the µ-scale, modularity, and accessibility for *in situ* analysis make our OoCs amenable for parallelization, high-content-screening, and plug&play connection to multi-organ-chips. These aspects paired with the biological complexity of the OoCs pave the way for applications in drug development, personalized medicine, toxicity screening, and mechanistic research.

Presentation: Oral

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Fluidic absorption drives cystogenesis in a human organoid-on-a-chip model of polycystic kidney disease

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Polycystic kidney disease (PKD) is a common genetic disorder in which tiny tubules in the kidneys and other organs expand to form fluid-filled cysts. PKD is commonly associated with mutations in *PKD1* and *PKD2*, but the function of these genes at the molecular level remain unclear, as do the mechanisms that drive cyst formation. To establish an organoid model of this disorder, we applied CRISPR gene editing to create loss-of-function mutations in *PKD1* or *PKD2* in human pluripotent stem cells, and differentiated these into human kidney organoids over a period of 26 days. PKD organoids formed cysts from kidney tubules at a ~15-fold higher rate than non-mutant isogenic control organoids cultured under identical conditions. To understand how flow affects PKD in organoids, we designed a microfluidic system that allows for live imaging of kidney organoids during the early stages of cyst formation. Organoids with small cysts were transferred into gas-permeable, tissue culture-treated polymer flow chambers, which were optically clear and large enough to comfortably accommodate organoids and cysts. PKD and isogenic control organoids were subjected to fluid flow with a wall shear stress of 0.2 dynes/cm^2, which approximates physiological shear stress within kidney tubules *in vivo*. In these devices, cysts in PKD organoids increased in size rapidly under flow (change in area of ~20,000 μm^2/h, or ~160 μm/h in diameter), compared to non-cystic compartments within these organoids, or isogenic control organoids lacking PKD mutations, which did not swell appreciably. A diffusive static condition could partially substitute for fluid flow, implicating volume and solute concentration as key mediators of this effect. Surprisingly, cyst-lining epithelia in organoids polarized outwards towards the media, arguing against a secretory mechanism. Rather, cyst expansion was driven by rapid glucose transport into cyst-lining epithelia and their lumens, which could be blocked pharmacologically to prevent cyst growth. We further demonstrated evidence of glucose absorption by cyst-lining epithelia in a mouse model of PKD *in vivo*. Collectively, these studies reveal that absorption can mediate PKD cyst growth, and PKD cystogenesis may be agnostic to epithelial polarity, with important implications for disease mechanism and therapy development.

Presentation: Oral

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A fully automated high-throughput platform for liver organoid differentiation from patient-derived induced pluripotent stem cells and drug screening applications

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Human liver organoids (HLOs) developed from patient-derived induced pluripotent stem cells (iPSCs) have shown to re-create the architecture, physiology, and pathophysiology of human liver in remarkable detail (Takebe et al., 2013; Ouchi et al., 2019; Shinozawa et al., 2021). They hold a great promise to transform drug development, precision medicine, and ultimately, transplantation-based therapies for end-stage diseases. Although the biological complexity of organoid cultures is a strength it also poses a significant challenge for automation and image-based screening applications hampering their use in industrial scale compound testing.

Here, we present a fully automated, HTS-compatible workflow for generation, maintenance, and optical analysis of HLOs in 96 well hydrogel-based microcavity arrays (Gri3D, Sunbiosciences). The formation of HLOs by guided aggregation of single cell progenitors in microcavity arrays resulted in organoids with highly homogeneous morphology, size, gene expression, cellular composition, and structure. The entire workflow from organoid generation to downstream analysis can be performed automatically with the use of liquid handler, robotic arm and high content confocal imaging system, enhancing the intra- and inter-batch reproducibility. The resulting HLO screening platform allows evaluation of drug effects at the single cell and single organoid level in a fully automated HTS workflow, including multiplexed assessment of liver health and function via high content image analysis and quantification of soluble liver injury biomarkers. This scalable HLO culture technology brings us one step closer to realize the full potential of organoids in drug development and precision medicine.

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Presentation: Poster

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Self-rolling multicellular constructs for tissue engineering

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We present a novel strategy for the development of 3D human arteries comprised of multiple cell layers by harnessing innovative strain engineering of a silicon oxide bilayer. As compared with endothelial cells in 2D culture, cells in the 3D constructs demonstrated improved cell viability and nitric oxide production.

Introduction: Arterial resistance vessels control flow to vascular systems. The structure, mechanics, and function of these blood vessels are critically important to our understanding of the mechanisms of cardiovascular disease pathogenesis. Additionally, mimetic approaches are needed to improve the study of cardiovascular disease, because models based upon rodent vascular cells and vascular responses in standard culture containers do not accurately recapitulate the infrastructure of human microvessels.

Materials and methods: Here, we describe novel, highly parallel, mass biofabrication of biomimetic tubular vessel constructs composed of biocompatible scaffold materials silicon oxide bilayers fabricated by photolithography and thin film deposition (a). The scaffold is constructed from essential components of human arteries, including pulmonary microvascular endothelial cells (HPMEC), extracellular matrix components including fibronectin and laminin, and human pulmonary artery smooth muscle cells (HPASMC). These constructs had an average diameter of 300 microns, and lengths of 1 mm to 3 mm.

Results and discussion: Cells were incorporated into these constructs in patterns that mimicked the layering and relative alignment observed in human small muscular pulmonary arteries. Finite Element Analysis showed that the ultra-thin cylindrical scaffold materials used here could be displaced by the cells in these biomimetic vascular walls in a manner similar to displacements of thicker materials composed of softer materials. HPMEC in these biomimetic vessel constructs produced more nitric oxide and demonstrated higher phosphorylation levels of the nitric oxide signaling protein eNOS as compared with equal numbers of cells grown on flat surfaces. HPASMC were aligned at tunable angles that mimicked *in vivo* organization using biomolecular micropatterning. High-resolution cellular imaging in these large organoid models was achieved using approaches relevant for many tissue engineering products.

Conclusions: Biomimetic vessels produced in this study exhibited improved endothelial functionality together with multi-cellular layering and anatomically accurate cellular alignment and provide a platform for investigation of microvascular function.

Presentation: Poster

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Organ-on-chip technologies in skin research, a reliable alternative to animal testing for cosmetic and pharmaceutical industries

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The reduction and replacement of animal models is the core of ethical innovation since the last few years. Novel technologies for cost-effective screening of chemicals, ingredients, drugs, and cosmetic formulations are in high demand because of the global need for reliable alternatives to animal models.

At Revivo Biosystems, we developed a microfluidic platform system for culture and testing of tissue biopsies and engineered 3D tissue equivalents to address these challenges. It mimics the native skin microenvironment realistically. When used with explants, our system enables multiplexed, automated skin permeation testing in a flow-through design, at a fraction of the material cost of traditional systems. We demonstrated its higher precision, sensitivity, and cost efficiency when using skin and oral mucosa substitutes, equivalents, and explants compared to existing technologies.

Our 3D cell cultures method optimized for our organ-on-chip systems enables *in situ* permeability, toxicity and efficacy testing, minimizing the risks associated with manual handling of the tissue. We demonstrated that our microfluidic approach and culturing technique improved epithelial morphogenesis and differentiation of full-thickness human skin and oral mucosa equivalents, ultimately favoring an enhanced synthesis of basement membrane proteins and barrier function.

With our organs-on-chip system, it is possible to determine the effects of any compounds on explants or 3D cell cultures no matter how they are applied (topically or systemically). Besides permeation assays, the system allows to predict if a compound induces irritation, inflammation, protective effects to different types of stress or if it changes the kinetic of the formation of the epithelium itself without the involvement of animals at any level. The efficiency of different formulations for the same compound can now be determined more easily and more accurately than ever before.

These scalable systems are amenable to automation of tissue culture and testing protocols, for drugs and cosmetic screening and toxicological applications.

Presentation: Poster

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A 3D alveolar *in vitro* construct for the prediction of chemical respiratory sensitizers

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The increasing prevalence of chemical respiratory allergic diseases resulting in high morbidity and mortality deems necessary a precise classification and labelling of chemicals inducing respiratory sensitization, to ensure the hazard is communicated and allow the safe handling and use. The immunological mechanisms underlying the development of respiratory sensitization are not fully understood, and the available new approach methodologies (NAMs) for identification of skin sensitizers fail to categorize chemicals as respiratory or skin sensitizers. Therefore, dedicated test systems for the respiratory tract are required to predict the sensitizing potential of a chemical.

In this study, we evaluated the performance of a 3D alveolar *in vitro* test system for the prediction of respiratory sensitization. The test system is built on microporous membrane inserts using epithelial, endothelial and monocytic cell lines. The physiologically relevant architecture of the test system favors the development of a tissue-like microenvironment by cell-to-cell direct communication and indirectly through the secreted messenger molecules. Additionally, it facilitates exposures at the air-liquid-interface (ALI).

A panel of test items comprising respiratory sensitizing chemicals and non-sensitizers was used to evaluate the performance of the test system to correctly identify sensitizers. Cell viability was determined as a measure of metabolic activity in the resazurin assay 24 h post exposure at ALI, and the dose-response curves were modelled for the apical, basolateral compartments, and the complete test system. The test system was afterwards exposed to a test item dose leading to a cell viability of at least 75% and the expression of CD54, CD86 and TSLPr cell surface markers was measured by flow cytometry.

The obtained data show that the test system has the potential to discriminate respiratory sensitizers from non-sensitizers. In addition, tested pro-haptens were correctly identified as respiratory sensitizers, chemicals reported as false negatives by other NAMs for respiratory sensitization evaluation.

Presentation: Poster

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Human on a chip systems applied to neurodegenerative and rare diseases

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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, CIDP, MMN, Myasthenia gravis, as well as its application to opioid overdose and recovery 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 where 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. 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. We will also describe a multi-organ innate immune system that was able to reproduce the pro-inflammatory and restorative phenotypes from macrophages. In addition, this talk will also give results of six workshops held at NIH to explore what is needed for validation and qualification of these new systems.

Presentation: Oral

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Biofabrication of long-lasting perfusable vascular tissue on chip

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Formation of functionally matured blood vessels is one of the major restrictions to size and complexity of engineered tissue structures in microphysiological systems (MPSs). Thus, the increasing demand for more comprehensive organ-on-chip models is fundamentally intertwined with vascularization and proper perfusion of the organs. However, in-lab engineering of microvascular tissue, with physiologically relevant perfusion capabilities, requires thorough understanding of the physics underlying how network morphology correlates with fluid flow distribution. 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 to a more organ-specific structure which better meets metabolic needs of the tissues. While vessel remodeling has long been found crucial to functionality of the vascular system in terms of proper distribution of blood, the matter of how differential vessel morphologies affect the overall vessel transport properties remains to be fully elucidated yet. We have previously shown that vessel network density can negatively affect the efficiency of blood distribution in murine retinal vasculature. Our coupled *in vivo* and *in silico* network and flow analyses showed that, vessel network complexity negatively affects blood perfusion efficiency and tissue oxygenation during neovascularization. These results, contradicting the conventional thinking about how branching density correlates with perfusion efficiency, 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 effect of fluid flow on shaping the vessel network architecture. Our model comprises human primary endothelial and stromal cells and exhibits long-lasting perfusability. Here, we present our current effort to implement a non-invasive imaging method for real-time analysis of microvascular growth, and to quantitatively investigate the dynamic network remodeling under flow. Results of our analyses will contribute to the next generation of vascularization platforms for MPSs as well as models for in-depth studying of vessel morphogenesis under flow.

Presentation: Poster

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A 3D shell MEA platform for brain organoids

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Multielectrode arrays (MEAs) have revolutionized *in-situ*, high spatiotemporal resolution electrophysiological recording and stimulation of neural and cardiac cell cultures. There are many commercial 2D MEA systems, which has been used for monolayer cultures, but they have limited contact area with organoids that have pronounced 3D shapes. This low contact between the recording and organoid surface can reduce signal to noise and limit 3D spatiotemporal interrogation. Thus, development of new devises allowing electrophysiological recording in 3D is needed. We developed self-folding 3D MEAs that can fold around organoids and permit 3D recording. We used induced Pluripotent Stem Cell-derived brain organoids at different stages of differentiation and showed the tunability of the 3D shell for different brain organoid sizes with programmable folding propensity. These 3D shell MEAs are optically transparent polymer devices patterned with metal/ conductive polymer electrodes, and they are compatible with prolong live cell recording and optical / fluorescence imaging. Here we describe fabrication, material, and operative features of the platform and discuss live-cell recording results. Importantly, we observe improved 3D electrophysiology recordings as compared to conventional 2D MEA recording. Our findings suggest that 3D shell MEAs could offer unprecedented capabilities for 3D spatiotemporal recording with good tunability, spatially resolved signals all over the 3D shape of the organoids, and long-term stability.

Presentation: Poster

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Integrated human intestine-liver-on-a-chip to elucidate liver injury induced by free fatty acid receptor 1 agonists

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Over the past years, many agonists for free fatty acid receptor 1 (FFAR 1), also known as GPR40, have been developed for the treatment of type 2 diabetes mellitus. Fasiglifam (TAK-875) was a promising candidate, however, in clinical phase III it had to be terminated due to liver safety concerns. Even though many *in vitro* and *in vivo* studies have been conducted, the exact mechanism of liver toxicity is not elucidated yet.

Here, we used a two-organ chip with a co-culture of human small intestinal epithelial cells and primary human liver microtissues to mimic an oral substance administration and close *in vivo* situation. In long-term experiments over a period of 14 days with repeated dose substance exposure, we investigated the mechanism of toxicity of Fasiglifam and a competitor compound developed by Boehringer Ingelheim GmbH & Co. KG.

In this study a complex analysis of cells and culture medium will be presented, including biomarker release, high-content live and antibody staining, and next-generation sequencing that led to the identification of different phenotypes of drug-induced liver injury.

Our study demonstrates the potential of multi-organ chips to identify drug induced toxicities and the benefits of microphysiological systems in preclinical studies for retrospective mechanistic investigations of drug effects.

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Developmental neurotoxicity of novel organophosphorus flame retardants using 3D hiPSC derived brain organoids as an MPS

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The use of MPS in neuroscience is essential to reproduce the central nervous system's (CNS) architecture, development, and function. This study aimed to evaluate potential of organophosphorus flame retardants (OPFRs) to induce developmental neurotoxicity in a human IPSC-derived brain organoids). The model consists of different cell types of the CNS such as neurons, astrocytes, and oligodendrocytes and has shown to be relevant for key cellular processes involved in neurodevelopment, including proliferation, differentiation, apoptosis, synaptogenesis, myelination, intracellular signaling, and network formation. OPFRs are the new replacement of phased out polybrominated FRs and ubiquitous FRs in consumer products, including children's products such as toys. They are suggested to be developmental neurotoxicants and might contribute to the increase in the prevalence of neurodevelopmental disorders. Brain organoids were exposed for 7 days to Triphenyl phosphate (TPHP: 1 μM, 10 μM, 20 μM), Isopropylated phenyl phosphate (IPP: 1μM, 10 μM, 20 μM) at 8 weeks of differentiation. Non-cytotoxic concentrations were determined by no significant difference in the percentage cell viability of the brain organoids exposed to these OPFRs vs. solvent controls [dimethyl sulfoxide 0.1% (v/v)] using resazurin assay. qRT-PCR was performed for selected non-cytotoxic concentrations to evaluate gene expression of markers for neurons (Btub3), synapse formation (Syn1), proliferation (Ki67), and oligodendrocytes (PLP1, CNP). Several of the genes evaluated had altered expression after exposure to these OPFRs and indicate developmental neurotoxic effects. Specifically, the oligodendrocyte markers (CNP and PLP1) and marker for synapse formation (Syn1) were affected with upregulated (CNP and PLP1) and downregulated (Syn1) expression in a dose-dependent manner. Immunohistochemistry and z-stack confocal imaging are currently being performed to confirm these effects.

These results indicate that synapse formation and oligodendrocyte differentiation and maturation are affected by exposure to these OPFRs in concordance with previous studies using an *in vitro* testing battery to assess developmental neurotoxicity.

Presentation: Poster

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Human biomimetic microphysiology systems (MPS) recapitulate the liver acinus for mechanistic, drug discovery/development and preclinical trial studies

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Many MPS experimental models of the liver have been developed to meet the "fit for purpose" needs in industry and basic research. We have developed two structured MPS of the human liver acinus using both primary human cells and iPSCs under flow stimulation: The liver acinus MPS (LAMPS) creates the spatial relationships and communications between hepatocytes, stellate cells, space of Disse, liver specific endothelial cells (LSECs) and Kupffer cells with the option of adding more immune cells. The LAMPS is a simple, relatively inexpensive/chip and has demonstrated excellent reproducibility in other laboratories. The vascularized, liver acinus MPS (vLAMPS) contains the same cells with the separation of the hepatic chamber and the vascular channel to allow the addition of drugs, blood born factors and cells into the model through the vascular channel.

We have applied the LAMPS and vLAMPS in five programs creating: 1) a progressive metabolic-dysfunction associated fatty liver disease (MAFLD) MPS model combined with quantitative systems pharmacology (QSP) to predict drugs for repurposing and as a starting point for novel drug development; 2) a liver-pancreatic islets MPS model to identify potential MAFLD liver specific factors (e.g., heptokines) that may promote downstream loss of β cell function and to serve as a drug discovery platform; 3) a liver niche for metastatic melanoma for drug discovery; 4) drug and biologics induced liver injury (DILI and BILI) MPS and 5) a preclinical trial for MAFLD based on patient cells with wildtype PNPLA3 and the I148M variant in the patatin-like phospholipase domain-containing protein demonstrated in patient populations where the variant has a strong association with progression of MAFLD.

An overview of the status of these programs will be presented with a discussion of the critical importance of using the BioSystics Analytics Platform (formally the MPS-Database) to create new knowledge.

Presentation: Oral

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Human kidney organoids demonstrate HDAC8 as a therapeutic target to attenuate EMT in kidney disease

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Epithelial-mesenchymal transition (EMT) is a cornerstone pathological cellular process in many disease states across many organ systems. Notably this occurs in the setting of cancer progression and fibrotic diseases. EMT has been demonstrated to play a key role in a variety of kidney diseases, influencing chronic disease progression, severity of symptoms and clinical outcomes. HDAC8 has been shown to be a potential therapeutic target in acute kidney injury settings, potentially modulating injury recovery and repair. The exact mechanism of HDAC8 as a therapeutic target in a kidney injury setting is unknown. However, recent studies in the breast cancer field demonstrate that HDAC8 inhibition plays a key role in down-regulating EMT, thereby preventing metastasis and disease progression. Here, using hiPSC derived kidney organoids, we demonstrate that in a renal injury model, HDAC8 knock out (KO) and chemical inhibition down-regulated EMT when compared to wildtype organoids. Key EMT and fibrosis gene markers such as aSMA, SNAI1, TGFb1, TWIST, ZO-1, CTNNB1, NFATC4, NFATC2IP, NFATC3, WNT1, ILK, FOXO1, and SRF were all down-regulated in the injured HDAC8 KO organoids. Immunofluorescent staining of injured HDAC8 KO kidney organoids for aSMA and fibronectin further corroborated this finding. To confirm HDAC8 as a therapeutic target, we used a known HDAC8 inhibitor, PCI-34051, to demonstrate reduction in fibrosis in injured wildtype kidney organoids. Data from this study was further analyzed within the MPS-Database (https://mps.csb.pitt.edu/) to compare with other MPS and *in vivo* models. With hiPSC kidney organoids as a demonstrated disease and toxicological model, this study lays foundational support for further functional and animal testing to confirm HDAC8 an important modulator of EMT in renal injury events.

Presentation: Poster

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Normalization of organ-on-a-chip samples for mass spectrometry based proteomics and metabolomics via dansylation-based assay

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Mass spectrometry based 'omics pairs well with organ-on-a-chip-based investigations, which often have limited cellular material for sampling. However, a common issue with these chip-based platforms is well-to-well or chip-to-chip variability in the proteome and metabolome due to factors such as plate edge effects, cellular asynchronization, effluent flow, and overall limited cell count. This causes high variability in label free quantitative workflows, thus masking true biological changes that occur within the system. Solutions to this problem have been approached via data processing tools and post-acquisition normalization strategies such as constant median, constant sum, and overall signal normalization. Unfortunately, these methods do not adequately correct for the large variations, resulting in a need for increased biological replicates. The methods in this work utilize a dansylation based assay with a subset of labeled metabolites that allow for pre-acquisition normalization to better correlate the biological perturbations that truly occur in chip-based platforms. This was performed in tandem with a proteomics pipeline employing a BCA protein assay to similarly achieve pre-acquisition normalization. The CN Bio PhysioMimix system was seeded with primary hepatocytes and challenged with VX after 6 days of culture, and the metabolome and proteome were analyzed using the described normalization methods. A decreased coefficient of variation percentage is achieved, more significant changes are observed through the proteome and metabolome, and better classification of biological replicates is acquired as a result of these strategies.

Presentation: Poster

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Using the GOFlowChip platform to investigate apoptotic cell death-dependent antigen sampling in the *Helicobacter pylori*-infected stomach

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Helicobacter pylori infects nearly half the world's population. Infections mostly remain asymptomatic, but the chronic gastric inflammation induced by *H. pylori* may lead to gastric cancer. Why the host response to this pathogen varies so significantly remains unclear, but antigen uptake and presentation by mononuclear phagocytes (MNPs), especially dendritic cells (DCs) is thought to play an important role. How MNPs take up antigen from the stomach lumen is still unknown. Some hypothesize MNPs directly interact with the epithelium, reaching dendrites through to phagocytose antigen, while others suggest uptake through gaps in the epithelium created during the turnover of short-lived gastric epithelial cells. Using *in vitro* cultures, we observed an increase in apoptotic cell death of *H. pylori*-injected human gastric organoids (HGOs) compared to uninfected organoids. Considering the observed apoptotic response, we investigated the function of apoptotic cell turnover to MNP sampling of *H. pylori*-infected gastric epithelium using the gastrointestinal organoid flow chip (GOFlowChip) platform. The GOFlowChip is optimized for co-cultures of 3-D gastrointestinal organoids with DCs and enables live confocal imaging. *H. pylori*-infected HGOs and DCs were co-cultured in the GOFlowChip, using Matrigel microbeads, or granular Matrigel, to support organoid culture and facilitate DC migration through pores in the gel. A fluorescent apoptosis reporter was added to observe and quantitate interactions of the DCs with apoptotic gastric epithelium in real-time for 20 hours. DCs in the GOFlowChip migrated rapidly towards infected HGOs in as little as 90 minutes. DCs surveyed the surface of both infected and uninfected organoids but preferentially accumulated around HGOs with increased apoptotic cell death. Phagocytosis of bacteria and apoptotic cell debris that had leaked from organoids was frequently observed, while strong adherence of DCs to the epithelial surface or subsequent uptake of cell material or bacteria from the organoid lumen was rare. Based on these observations, we propose that *H. pylori*-induced epithelial death may enhance antigen sampling in the gastric mucosa by increasing DC recruitment and antigen transfer across the epithelium.

Presentation: Poster

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Development of human bone metastases on a microscale chip

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Background: Bone metastases occur frequently in many solid tumors including prostate cancer (PCa), and are associated with poor patient outcomes. The bone metastatic niche contains multiple specialized stromal cells, in addition to immune cells and vasculature. Currently, pre-clinical models fail to replicate the tumor microenvironment in bone metastases, limiting our ability to evaluate treatment response or tumor progression. Here we have developed a microphysiological system (MPS) to model the complex multicellular niche of the PCa metastatic bone microenvironment.

Methods: PCa epithelial cells were isolated from prostatectomy specimens while mesenchymal stem cells (MSC) were isolated from bone marrow aspirates from patients with PCa. To generate osteoblasts, adipocytes and fibroblasts, MSCs were differentiated for 3 weeks in specific media formulations. Monocytes were isolated from peripheral blood from patients with PCa and further differentiated to M2 macrophages and osteoclasts. A high-throughput plate assay identified a media formulation that can support the growth of all of these highly specialized cell types. The bone metastasis chip is based on the LumeNEXT platform, where a mixed population of bone stromal cells and PCa epithelial cell spheroids are cultured in a collagen matrix, flanked by induced pluripotent stem cell (iPSC) derived endothelial cell lumens to provide vasculature. Cell viability was measured using Calcein and Ethidium homodimer staining. Retention of differentiated cell phenotype was evaluated by qPCR.

Results: To populate the bone metastasis chip, we differentiated adipocytes, osteoblasts, osteoclasts, fibroblasts and M2 macrophages and performed extensive media testing to identify a multi-phenotype media formulation that supported growth and phenotype retention of these cells. Bone metastasis chips were cultured for 14 days retaining > 80% cell viability. qPCR screening of adipocyte, osteoblast, osteoclast and MSC genes confirmed that their phenotype is retained in the bone metastasis chip. Mineralization of the matrix by osteoblasts was observed in the chip. PCa epithelial spheroids were introduced into the bone metastasis chip to model the solid tumor. They remained viable and continued proliferating for 14 days.

Conclusions: We have created a bone metastasis chip to model the PCa bone metastatic environment that could be used to investigate response or resistance to drug treatment.

Presentation: Poster

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Computational modeling of fluid dynamics and pharmacokinetics for human-on-a-chip system design

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Hesperos, Inc. aims to substantially reduce animal pharmaceutical testing and improve compound efficiency in preclinical trials to reduce late-stage drug failure by utilizing body-on-a-chip technology with human tissues coupled to bioMEMS devices to form Human-on-a-Chip®. This technology aims to recapitulate *in-vivo* physiological markers and outputs in an *in-vitro* system to reduce cost, improve pharmaceutical tests and improve patient outcomes. Hesperos, Inc. has developed a portfolio of serum-free and low volume multi-organ microfluidic systems that allow for non-invasive electrical and mechanical functional measurements on cardiac, neuronal, skeletal muscle, neuromuscular, and other tissues including functional barriers. The objective of Human-on-a-Chip technology is to develop *in-vitro* systems representative of *in vivo* organ or tissue physiology. This approach offers a platform that can imitate a drug's metabolic lifecycle (pharmacokinetics) and functional effects in the human body (pharmacodynamics) to conduct toxicology and efficacy studies for disease treatment. Toward this goal, Hesperos' multi-organ microfluidic systems feature recirculation of a low volume of medium using a pumpless design, enabling pharmacokinetic studies and organ-organ interaction. A major component in the engineering design of these microfluidic systems is the application of computational techniques and pharmacokinetic modeling strategies to predict drug fate in these devices. A combination of computational fluid dynamics (CFD) and other numerical methods are used to engineer flow rates, shear stresses, and pharmacokinetics including first pass metabolism and mixing rates using a multitude of flow schemes with dynamic rocking frequency, tilt angles and tilt orientation. This procedure allows for device extension to multi-organ, multi-level systems while minimizing development

costs. Several Hesperos systems are highlighted demonstrating the use of computational modeling in the engineering design of Hesperos' microfluidic Human-on-a-Chip devices. Additionally, pharmacokinetic predictive modeling of applied compounds in these systems is demonstrated.

Presentation: Poster

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Maximizing skeletal muscle adhesion to enhance human neuromuscular junction integrity and function

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We have developed Myasthenia gravis (MG) and Amyotrophic Lateral Sclerosis (ALS) patient-derived neuromuscular (NMJ) models on a BioMEMs platform to better understand the progression of ALS and possible causes for its correlated NMJ dysfunction. The co-culture models containing ALS human skeletal muscle (hSKM) or human motoneurons (hMNs) have shown significant reduction in NMJ formation, stability, and contraction fidelity, as well as increased fatigue index. The defects observed in NMJ systems with ALS-hSKM are relatively more severe than those with ALS-MNs, highlighting the importance of targeting hSKM in the protection of NMJ integrity in ALS. We have also developed an MG model and demonstrated all three mechanisms and are applying it to evaluate therapeutics.

One of the general limitations in micro-physiological systems is the difficulty of studying the effects of long-term drug exposure, as contractile cells can de-adhere from systems over time or with repeated testing. Understanding cell-extracellular matrix (ECM) interactions for tissue-specific and corresponding dynamic functional integration can help cells with attachment and intercellular communication. We have studied different mechanisms of skeletal muscle cell adherence and described how surface effects influence function in this model. By extending the longevity of muscle adherence, this work can pave the way for modeling muscle aging and its associated diseases *in-vitro*, as well as generating in-depth toxicology assessments for drugs requiring long-term treatment and ascertaining possible therapies for myopathies.

Presentation: Poster

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Pancreatic slices on a chip: A model to study beta cell neogenesis

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Functional long-term cultures of human pancreatic slices allow for the longitudinal tracking of β-cell regeneration from progenitor cells in healthy and diseased tissues. Current methods for long-term cultures of pancreatic slices utilize static cultures sitting atop an oxygen permeable membrane. Static cultures do not allow the continual removal of metabolites and require intensive user interaction to add different biologic interrogators. Sequential administration of interrogators along with continuous metabolite removal can provide a more physiologically relevant environment for pancreatic slice cultures in which we can study potential β-cell neogenesis in the pancreas. An islet MPS previously developed in our lab provided a distinct portfolio of capabilities not available in other platforms, specifically long-term culture, controlled and dynamic perifusion, *in situ* imaging, serial cell assessments during extended culture, ease of biochemical perturbation, and sample retrieval. Here, we sought to modify and extend the islet microphysiological system to develop a pancreatic slice on a chip model that is capable of sustained slice culture with concurrent fluorescence microscopy, allowing for serial visualization of cellular changes in response to biologic interrogators. To ameliorate movement artifacts that are evident under long-term imaging, a 3D printed bioinert anchor was developed to stabilize slices. The anchor design ensures that media moves freely across the slice while limiting the slice's ability respond to variations in flow. Media flow in our chip is generated by a constant-pressure pump that pressurizes media containers connected to the chip. In addition to media, biological stimulants used to interrogate the slices, such as glucose and carbachol, can be sequentially fed to the slices through a fluidic switch. The ability to sequentially stimulate the slices within a microscope will also allow for detailed Ca^{2+} imaging. To maintain slice viability and fluidic inputs within the microscope, a cell incubation chamber manufactured by the microscope manufacturer was adapted to have fluidic ports that could feed into the chip without breaking the sterility or function of the incubation stage. Our pancreatic slice on a chip allows for continuous visualization of β-cells, their neogenesis from progenitor cells within a physiomimetic environment and cellular signaling in response to stimuli.

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Modeling endometrial-immune crosstalk in micro-physiological systems

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Introduction: Endometrial and innate immune system crosstalk in patients with endometriotic disease is poorly understood. To investigate these interactions during an *in vitro* reconstruction of the menstrual cycle, we incorporated $CD14^+$ monocytes and endometrial stromal cells in a 3D synthetic hydrogel extracellular matrix co-culture model. We hypothesized that proliferation/clearance pathways would be differentially enriched between menstrual phases and between healthy and diseased patients. Recent efforts to transition such a co-culture into microphysiological systems demonstrate a comparable decidual response, suggesting that these co-culture models can be incorporated into more complex, physiologically relevant systems while maintaining stromal cell function.

Methods: Endometrial stromal cells were collected from uterine biopsies with (N = 3) and without (N = 3) endometriosis and cultured for 14 days in media containing either estradiol (E2) or E2 with medroxyprogesterone acetate (MPA) to induce decidualization. Stromal cells were labelled with CellTracker™ Red and co-encapsulated with CellTracker™ green-labelled $CD14^+$ monocytes inside 3D poly(ethylene glycol)-based extracellular matrix droplets. We then cultured the droplets in conditions mimicking the "proliferative phase" (E2 only), the "secretory phase" (E2 & MPA), or "menses" (MPA withdrawal) for 7 days. Time-course images were captured to monitor cell proliferation. Harvested cells were flow-sorted into stromal and immune populations for bulk RNA-seq analysis. Custom cyclin olefin copolymer (COC) microfluidic devices were fabricated to investigate time-course decidualization by measuring stromal cell prolactin secretion while embedded in PEG hydrogels.

Results: Enhanced monocyte proliferation was observed in the "menses" condition compared to the "proliferative" condition after co-culture with heathy stromal cells ($p < 0.05$); this increase was not reflected in co-cultures with diseased stromal cells. Monocyte maturation was inferred by projecting our monocyte data into a reference monocyte data set. We then observed that monocytes cultured with diseased stromal cells more closely matched GM-CSF/TGFβ/TNFα-stimulated monocytes compared to monocytes cultured with healthy stromal cells.

Conclusion: We found that healthy and diseased stromal cells differentially influenced monocyte differentiation and proliferation, with the most significant differences observed during the "menses phase". This study is the first to suggest that the diseased stromal compartment may weaken proliferation and influence the differentiation of circulating immune cells.

Presentation: Oral

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Building 3D models of human blood-retinal barrier-on-a-chip as a new platform for drug discovery

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Age-related Macular Degeneration (AMD) is a very common ocular disease which leads to blindness in elderly people. Like the Blood Brain Barrier (BBB) in the brain, the eye is a well-protected organ with two barriers; an inner BRB (iBRB) composed of specialized retinal vasculature protecting neural retina and an outer BRB (oBRB) composed of a retinal pigment epithelium-choroidal vessel system which protects retinal photoreceptors. The mechanism of the majority AMD (dry form) is not clearly understood; thus, no treatment has been approved. The cause of wet AMD (10% of all cases) is due to vascular leakage from the choroidal vasculature and neovascularization penetrating the RPE layer and causing RPE barrier breakdown. Currently intravitreal injection of anti-vascular endothelial cell growth factor (VEGF) drugs is the main therapy for neovascular AMD. Besides the invasiveness of the treatment procedure, the effectiveness of anti-VEGF in preventing the progression of AMD has been seen in only a portion of patients and vision recovery is also seen in only 30% of patients. The effect of anti-VEGF is temporary, but long-term use may cause chorioretinal atrophy as VEGF is an essential factor for endothelial cell survival. Traditional 2D models such as the RPE monolayer model or endothelial cell monoculture do not recapitulate the complex pathological process, such as the RPE-choroidal interaction and thus do not provide a versatile platform for new drug discovery. To overcome the limitation of traditional 2D models, we have built 3D models of oBRB and iBRB using Curiochips which supports 3D blood vessel networks and neighboring with RPE monolayer or co-culturing with other supporting cells. The key advantage of using Curiochips is that they are readily reformattable for high-throughput screening (HTS). We will discuss the utility of the BRB 3D model on Curiochips in understanding AMD disease and its progression, as well as its utility as a drug discovery platform for the development of new therapies beyond anti-VEGF.

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Precision printing multi-cell structures on chemically patterned microelectrode arrays for human-on-a-chip

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Hesperos, Inc. aims to substantially reduce animal pharmaceutical testing and improve compound efficiency in preclinical trials to reduce late-stage drug failure by utilizing body-on-a-chip technology with human tissues coupled to bioMEMS devices to form Human-on-a-Chip®. This technology aims to recapitulate *in-vivo* physiological markers and outputs in an *in-vitro* system to reduce cost, improve pharmaceutical tests and improve patient outcomes. Hesperos uses photolithographical patterning of surface chemistries to control the structure of cultures on bioMEMS devices such as microelectrode arrays (MEAs) recording functional electrical measurements such as electrical behavior of cardiac syncytia or networks of neuronal cells. The additional use of precision plating techniques further enables more complex structures between multiple cells types on these bioMEMS devices and allows improved utilization. We have developed a method to place cells affordably, quickly, and accurately onto the surface of bioMEMS devices such as microelectrode arrays, utilizing the Opentrons OT-2 pipetting robot to move and dispense cells directly onto the head of each electrode. This method offers many advantages, such as reducing the required number of cells as each dispensed drop is positioned precisely at the point of interest. This cell plating workflow can be semi/fully automated, requiring minimal human interaction and speeding up user workflows. This technology allows for the creation of mixed cultures in which each electrode head contains a different cell type, and their effects on each other can then be incorporated into the Human-on-a-Chip system. To this end, we demonstrated the ability to plate two distinct populations cells onto separate electrode heads of an MEA with survivability and structural integrity maintained over multiple days.

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Leveraging organ-on-chip platforms to study gut dysbiosis-breast tumor relationships

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Changes in gut microbiome composition have been correlated with multiple cancer types, ranging from local colon cancers to tumors in more distal organs. Various methods of action by which gut microbes may modulate tumorigenesis in organs such as the breast have been proposed including production of pro- and anti-inflammatory molecules and the metabolism of estrogenic compounds. Current models are limited in their capacity to study how the microbiome may drive tumorigenesis and how tumors might alter microbiome composition. Better understanding of this relationship could improve intervention strategies for high risk patients and treatment outcomes for those who develop cancer. We have developed two organ-on-chip platforms, one using oxygen impermeable plastic to culture cancer-associated gut microbes and another for breast cancer tissue models such as spheroids. This two-chip system enables direct linking of organ models to isolate driver molecules responsible for the gut dysbiosis-tumor relationship. Work-to-date has demonstrated signs of early polarization in villus-like gut epithelium and co-culture with commensal bacteria, while the tumor chip enables maintenance of spheroid viability and apical-out orientation.

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Integration of a defined synthetic extracellular matrix with microfluidic devices for 3D perfused liver culture

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Introduction: Human liver models are needed that capture complex phenotypes involving inflammation, off-target effects of biologics, and immune cell trafficking. 3D models incorporating perfusable in non-PDMS materials have been slow to arrive – such as thermoplastics which reduce small molecule adsorption but lead to challenges in oxygen delivery. Further, fibrin gels for forming 3D vascular networks and Matrigel for 3D liver cell culture, have many shortcomings. Our previous work defined a synthetic polyethylene glycol (PEG)-based synthetic extracellular matrix (ECM) suitable for generation, expansion and co-culture of encapsulated human gut, endometrial, and pancreatic tumor epithelial organoids with immune and stromal cells (Hernandez-Gordillo et al., 2020; Below et al., 2022; Gnecco et al., 2021). Here, we describe steps integrating two complementary approaches towards building vascularized liver models: 3D perfused culture of liver spheroids in a synthetic ECM, and compact thermoplastic devices designed with on-board micropumps and pressure regulators to sustain flow and a desired pressure gradients in a tissue compartment suitable for inducing microvascular networks.

Methods: We conducted a semi-empirical screen of biochemical and biophysical parameter space for our synthetic ECM modified with integrin-binding and ECM-binding ligands, varying composition and mechanical properties in a systematic fashion. The hydrogels were initially incorporated in a pre-existing perfused MPS platform (Physiomimix, CN-Bio) and in our homegrown thermoplastic devices which allowed us to image cells during culture.

Results: Synthetic ECMs supported hepatocellular function and significant remodeling. Translation of network-forming phenotypes exhibited in free-swelling PEG gels was impaired, perhaps due to confinement, as thermoplastic devices that supported perfusable vessels in fibrin gels did not readily support perfusable vessels in PEG gels from co-cultures of endothelial cells and fibroblasts; studies are ongoing to define suitable regimes of gel composition and device operation (e.g., trans-tissue pressure drops).

Conclusions: These studies lay the foundation for integration of a fully defined synthetic ECM with an imaging friendly thermoplastic device towards a vascular perfused 3D liver model.

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Presentation: Poster

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Development of an *in vitro* 3D neuro-vascular model for Alzheimer's disease

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Alzheimer's disease (AD) is prevalent worldwide, with a rising rate of incidence as the population ages. The pathological hallmark of the disease is the deposition of amyloid-β protein (Aβ), namely amyloid plaques, in the brain which eventually leads to neuronal death through a pathogenic cascade (Sweeney et al., 2019). One of the key pathways for clearance of Aβ is via the blood brain barrier (BBB) which restricts the transport and clearance of Aβ peptides (Sweeney et al., 2019). It has been shown that the BBB is impaired in AD patients and that a dysfunctional BBB is a key factor in AD pathogenesis. It is also known that the presence of Aβ and its accumulation around the brain microvessels can impair BBB function. Recognizing the importance of this barrier in AD, we developed a microfluidic model to better understand Aβ accumulation in and clearance from the brain parenchyma. Combining our current BBB model and an earlier model for Aβ interactions with an endothelial monolayer (Shin et al., 2019; Hajal et al., 2022) incorporating human wild-type and AD neurons in suspension or as neurospheres, we have developed a model that more accurately recapitulates conditions similar to those found *in vivo*. With this model, we are able to capture the aggregation, distribution and clearance of Aβ but also image the Aβ plaques explicitly and quantify its effect on barrier function over periods of over one month.

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Presentation: Oral

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Engineering physiologically relevant 3D models of the intestine via organoid morphogenesis in synthetic hydrogel

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The 3D architecture of the native intestine, with its separate stem cell (crypt) and differentiated cell (lumen/villus) compartments, is important to maintain proper function of the organ. The few *in vitro* models of the intestine that have attempted to recapitulate this structure have relied on templated matrices and complex growth factor delivery schemes to maintain a proliferative stem cell compartment over the course of an experiment. Our approach instead relies on principles of emergent behavior and leverages the advantages of a synthetic PEG hydrogel matrix to control the biophysical and biochemical microenvironment. This synthetic PEG hydrogel has been optimized for intestinal epithelial organoid culture (Hernandez-Gordillo et al., 2020). In this work, we have carried out a semi-empirical screen of biophysical and biochemical microenvironment cues required to elicit features of the crypt-lumen structure from primary human intestinal epithelial cells cultured alone or with primary human intestinal fibroblasts. We have demonstrated that human duodenal organoids embedded in a poly(ethylene glycol) based synthetic hydrogel undergo morphogenesis to generate multiple complex structures, including a textured, apically-accessible epithelial monolayer and projections up to 500 µm in length. In these projections, the non-embedded portion demonstrated reverse apical-basal polarity relative to the embedded portion. We can control the generation of these structures by modulating hydrogel matrix stiffness, organoid density, and co-culture with intestinal fibroblasts. EdU assays indicate epithelial cells in this system remain viable and proliferative for 28 days in static culture. Together, these results demonstrate a 3D *in vitro* model supportive of long-term intestinal epithelial-stromal co-culture and with a fully defined microenvironment suitable for mechanistic investigations; studies further applying this *in vitro* model to investigate mechanisms of tissue morphogenesis are ongoing.

Reference

Hernandez-Gordillo, V. et al. (2020). *Biomaterials 254*, 120125.

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Microvascular model incorporating cancer-associated fibroblasts and immune cell perfusion

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Microphysiological models of the tumor microenvironment have become increasingly sophisticated, but studies using T cells remain difficult due to the need to avoid an allogeneic response. We have developed a microvascular model for T cell perfusion that addresses these challenges using cancer-associated fibroblasts (CAF) and T cells from patients in combination with endothelial cells engineered to eliminate expression of major histocompatibility complex class I.

To build the model, tissue specimens from thyroid and oral cavity cancers were dissociated, and CAF and T cells were isolated and expanded. CAF or normal fibroblasts (lung, dermal, or thyroid) were embedded in microfluidic devices, either with fluorescent HUVEC applied as a monolayer on the gel face to assess sprouting or co-mixed within the gel for vascular networks. Endothelial cells sprouted more readily into gels with CAF than those containing normal fibroblasts, with 2.35-fold higher sprouting area ($p = 3 \times 10^{-18}$). Analysis of secreted factors revealed that CAF produced significantly greater VEGF and CCL5 than normal fibroblasts, and CCL5 was positively correlated with sprout area ($p = 0.003$, $R^2 = 0.74$). CAF supported the generation of self-assembled microvascular networks, which were perfused with patient-matched T cells. Observation using confocal microscopy showed that 70% of the retained T cells were bound to a vessel lumen, 17% were physically trapped in small diameter vessels, and 13% extravasated to the interstitial space.

This platform creates a comprehensive *in vitro* system recapitulating the tumor microenvironment that allows T cells to be introduced via circulation through self-assembled vascular networks in microfluidic devices. In addition to aiding our understanding of the influence of CAFs on the vascular TME, ongoing work incorporates patient-derived organotypic spheroids to observe T cell killing and serve as a platform for immunotherapy testing and biomarker identification.

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Presentation: Oral

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IQ MPS affiliates: Accelerating the development and adoption of MPS models in industry

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The IQ MPS is a collaboration of pharmaceutical and biotechnology companies created as an Affiliate within the International Consortium for Innovation and Quality in Pharmaceutical Development (also known as the IQ Consortium). The IQ Consortium is a leading science-focused, not-for-profit organization with a mission of advancing science and technology to augment the capability of member companies to develop transformational solutions that benefit patients, regulators and the broader R&D community. The IQ MPS Affiliate was established to provide a venue for appropriate cross-pharma collaboration and data sharing to facilitate the industry implementation and qualification of MPS models. The IQ MPS Affiliate is devoted to raising awareness, advancing the science, and supporting the implementation of MPS in drug discovery.

This talk will provide an overview of the IQ MPS Affiliate organization, its outreach, and goals. The current activities of the six working groups within the IQ MPS Affiliate will be described in detail including our upcoming organotypic manuscripts, outreach with regulatory bodies, and joint precompetitive pilot projects in gut and kidney MPS. In general, this cross-pharma collective hopes to boost the adoption of MPS by industry, standardize outputs to clear regulatory hurdles, and help realize the potential of these models to reduce animal use while making drugs safer and more effective. We welcome engagement with our group to realize this shared vision.

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Engineering physiologically relevant models of hepatic insulin resistance

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An estimated 70% of individuals with Type 2 Diabetes also suffer from nonalcoholic fatty liver disease (NAFLD). Insulin resistance is pathologically fundamental to both NAFLD and T2D, however the mechanisms of insulin resistance linking these complex diseases remains incompletely understood. Microphysiological systems provide an avenue to simulate disease parameters in the context of human biology, however current *in vitro* models of hepatic insulin resistance often include nutrient and insulin concentrations orders of magnitude above *in vivo* levels. Here, we established an *in vitro* model applying the CN Bio Physiomimix platform to culture primary human hepatocytes and non-parenchymal cells in physiologically relevant media conditions simulating both baseline physiological and T2D conditions. Albumin secretion and cytochrome p450 assays indicate hepatocytes remain functional over two weeks in culture, while cytokine induction indicates responsive Kupffer cells. Results further show hepatocytes cultured in physiological conditions maintain insulin responsiveness after 12 days in culture, measured by glucose output following dose response of insulin stimulation. Conversely, cells cultured in T2D media conditions have significantly attenuated response to insulin stimulation compared to the physiological condition. Measuring insulin clearance from the media also showed reduced clearance over time for the T2D conditions. Together, these results demonstrate a 3D *in vitro* model supportive of modeling hepatic insulin resistance through culture with insulin concentrations found in the portal vein of T2D patients. Studies further applying this *in vitro* model to understand insulin resistance mechanisms are ongoing.

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Synaptogenesis assay for developmental neurotoxicity testing in a human 3D brain model

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The rapid increase in the prevalence of many neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD) is a major public health concern. Synaptogenesis is one of the key features of neural development and one of the important events perturbed by ASD. Thus, early developmental disruptions to this process have been hypothesized to be one of the possible mechanisms behind the emergence of NDDs.

Current *in vivo* animal tests for developmental neurotoxicity (DNT) are associated with high costs, ethical concerns and technical difficulties and so are prohibitive for routine DNT chemical screening. However, emerging 3D organotypic cultures of microphysiological systems are a promising replacement.

Our group has previously generated a human induced pluripotent stem cell (hiPSC)-derived brain organoid model. To establish a robust high-throughput model for synaptogenesis, we took this model and generated hiPSCs with a green fluorescence protein (GFP) tag under the synaptic marker gene, synaptophysin, using CRISPR-Cas9 technology. Homozygous iPSC-GFP-Syn clones were then selected and quality controlled. They were then differentiated into neural progenitor cells (NPCs) and plated on a gyratory shaker to generate 3D organoids. Immunocytochemistry showed slight GFP expression at 2-weeks of differentiation, with a continuous increase in GFP signal over further 10-weeks of differentiation. Colocalization with synaptic markers (Synapsin1 and Synaptophysin) and the formation of complex cellular histoarchitecture were also confirmed. We are currently performing testing with model DNT toxicants (Lead and Chlorpyrifos). As a next step, red fluorescence protein (RFP) will be introduced into the iPSCS-GFP-Syn line to tag the postsynaptic marker PSD95. Assessment of GFP/RFP colocalization in iPSC-GFP-Syn-RFP-PSD95 organoids will allow quantification of active synapsis using high-content imaging in live cells.

This new *in vitro* assay could reduce the burden of DNT animal testing while providing human-relevant data for risk assessors to better control chemical exposures and improve human health outcomes.

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Validating multiple lines of hiPSC-derived cerebral organoids against a murine model and establishing a proxy for age relative to human brain development

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Research into neurodevelopmental diseases (NDDs) is impeded by a dearth of valid pre-clinical models, in which the majority utilize rodents despite significant species-specific differences to humans. Thus, human induced pluripotent stem cell (hiPSC)-derived cerebral organoids emerge with tremendous potential to produce more human-relevant results; however, important limitations remain, notably in variability due to heterogeneity between donor lines, and the inability to approximate their relative age to the human brain. Additionally, ectodermal differentiated organoids lack microglia, which are crucial for neurodevelopment. Herein, we describe our recent work to assess these barriers and optimize our current model, in relation to key events of neurodevelopment. As a multitude of NDDs involve aberrations in glutamatergic signaling, we examine expression patterns of N-methyl-D-aspartate receptor (NMDAR) subunits, since a shift in GluN2B-GluN2A timing often underlies cognitive dysregulation in disease states. Organoids were cultured from two lines (A and B, including a subset with incorporated microglia) with samples taken every 2-weeks for longitudinal characterization by qRT-PCR for genes encoding GluN2A, GluN2B, NeuN (mature neurons), Ki67 (proliferation), and pre- and post-synaptic markers (synapsin1, SAP102, PSD95). In a parallel study, mice were sacrificed during their comparable period of neurodevelopment (PND5-14). We found differences in expression patterns between the lines, with only line A displaying consistencies to expected human neurodevelopment; specifically, NeuN, Ki67, synapsin1, and GluN2A-PSD95 continually increased, whereas GluNB-SAP102 decreased at 6-weeks. In murine samples, the GluN2B-GluN2A shift occurred at PND11, whereas synapsin1 decreased after PND10 in the hippocampus but not pre-frontal cortex. In sum, A line-derived organoids best recapitulated human neurodevelopment, with GluN2B-GluN2A patterns also offering a proxy for age correlation, namely that 6-week organoids may equate to a 20-50 post-conception week fetus. Continued comparisons between organoid and animal models not only serve to substantiate their use as a replacement method, but also ability to advance translational medicine.

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Differential impacts of early and late exposure to heavy metals and metal mixtures in a human cerebral organoid model

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Heavy metals are among the most concerning toxicants provoking dysfunction in human brain development. Exposure to heavy metal mixtures via drinking water represents one pathway of concern for pregnant mothers and young children, yet the effects of these exposures remain poorly understood. We used an iPSC-derived human cerebral organoid model to characterize the effects of Pb, Cr, Cd, and As alone and in combination at human relevant exposure concentrations during early (0-4) and later (8-12) weeks of neuronal differentiation and maturation using a standard developmental neurotoxicity assay battery.

Little effect was observed in differentiation, gene expression, oxidative stress response, neurite outgrowth, neurotransmitter generation, or methylation indicators following exposure from 0 to 4 weeks post-differentiation (wpd). In contrast, individual heavy metal exposure from 8 to 12 wpd elicited a unique profile of effects, though all commonly interfere with ROS production or defense and neurotransmitter formation in some capacity. NESTIN, a neural progenitor and radial glia marker, was downregulated and the morphology of Nestin-positive cells changed, while mature astrocyte marker S100B was also downregulated across all toxicants. Consistent with known effects of Pb exposure, Pb reduced neurite density and the expression of both dendritic marker MAP2 and pre-synaptic SYN1 following late, but not early, exposure. In contrast, Cd, Cr, and As exposures yielded more dense but erratic neurite outgrowth, while similarly reducing MAP2 and SYN1 expression. Late Pb exposure also increased intracellular glutamate and acetylcholine, decreased GABA, strongly reduced cystine, a substrate of glutathione (GSH), and reduced the ratio of GSH to GSSG, indicating the initiation of antioxidant response. Pb also altered S-Adenosyl Methionine and S-Adenosyl Homocysteine levels. Interestingly, in most cases where an effect was observed for individual heavy metal exposure, a lesser effect or no effect was observed for metal mixtures. These results demonstrate the potential for heavy metal induction of developmental perturbations at concentrations are currently believed to be health protective, and indicate that disruption is dependent on exposure timing. Attenuation of these effects in mixture exposures was unexpected, and has been verified in duplicate experiments. Additional investigations are necessary to elucidate the mechanistic basis for these effects.

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Biodistribution and PK modeling of a multi-organ human-on-a-chip system consisting of a GI tract, blood brain barrier and neurons

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Hesperos, Inc. aims to minimize animal testing and to improve compound testing efficiency in preclinical trials to reduce late stage drug failure by utilizing body-on-a-chip technology with human tissues coupled to bioMEMS devices. The objective of body-on-a-chip technology is to develop *in-vitro* systems representative of *in vivo* organ or tissue physiology. This would offer a platform that imitates a drug's metabolic lifecycle (pharmacokinetics) and functional effects in the human body (pharmacodynamics) to conduct toxicology and efficacy studies for disease treatment. Hesperos, Inc. has developed a portfolio of serum free, low volume, multi-organ microfluidic systems in which electrical and mechanical function can be measured non-invasively from cardiac, neuronal, skeletal muscle and barrier tissues like blood brain barrier (BBB) and gastrointestinal tract (GI) tissues. A multi-organ (3 organ) BioMEMS human on a chip system consisting of a GI tract barrier, BBB and CNS was developed and characterized for drug kinetics in the system. An *in vitro-in vivo* extrapolation (IVIVE) approach was applied to determine the continuous pharmacokinetic profiles of each drug and linking together with the pharmacodynamics of neuronal functionality. This approach serves 3 fundamental necessities a) *in-vitro* to *in-vivo* translation b) computing a required dose to achieve a desired PK profile, and c) establishing continuous PKPD relationships. The multicompartment PK model was employed to compute the uptake for the GI tract and the access to the target tissue site by evaluating penetration across the BBB. Population PK (pop-PK) models were developed to characterize experimentally determined concentration- time profiles of drugs following an intravenous or oral dose. These pop-PK models consisted of three interconnected compartments. Following either per oral (PO) or intravenous (IV) dosing, drug distribution through the system into the blood from the GI-tract and from the blood to the brain was characterized by the intercompartmental clearances and CL(GI,blood) and CL(blood, brain). Drug specific binding of the compounds to the system was accounted for and estimated by respective volume of distribution. The model was used to predict the required dose to achieve the desired target concentration-time profiles at the neuronal MEA established from the single organ dose-response.

Presentation: Oral

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Biophysical and biochemical determinants of angiogenesis into synthetic PEG hydrogels from perfusable microvasculature

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Background: Sprouting angiogenesis, the formation of new blood vessels from pre-existing vasculature, is essential in tissue regeneration and the progression of various diseases. Most *in vitro* angiogenesis models rely on natural matrices such as fibrin, collagen, and Matrigel, which all vary lot-to-lot, degrade relatively quickly, and are not ideal for supporting specialized cell types into which angiogenesis of perfusable vessels is desired, such as certain organoids. Here, we build upon well-established protocols for creating stable, perfusable microvascular beds in microfluidic devices, wherein endothelial cells and fibroblasts undergo vasculogenesis in a fibrin matrix. We focus on analyzing the growth of perfusable vessels from this vascular bed into a degradable, synthetic polyethylene glycol (PEG)-based hydrogel, which is functionalized with integrin-binding and matrix-binding peptides in order to encapsulate cells requiring vascular support.

Methods: Human umbilical vein endothelial cells (HUVECs) and supporting fibroblasts were mixed in fibrin and allowed to self-assemble into perfusable microvascular networks (MVNs), inside the central channel of a PDMS-based microfluidic chip that allows for later insertion of an additional ~2 mm cell-gel construct. After MVN formation, 750 µm lung fibroblast spheroid aggregates were encapsulated in PEG and loaded into compartments within the central channel.

Results: Over 7 days of co-culture, we observed that MVNs maintained perfusability while angiogenic sprouting developed across the fibrin-PEG interface and penetrated into the spheroids. Through systematic variation of the PEG hydrogel's biophysical properties, we demonstrated that lowering polymer concentration and crosslinker density resulted in increased angiogenesis, quantified by image analysis of sprouting area. From a molecular perspective, ongoing work involving previous hydrogel characterization studies suggests that adhesion ligand identity and density also play a key role in promoting spheroid compartment vascularization, which may be further enhanced by vasculogenesis of HUVECs suspended within the PEG hydrogel.

Conclusion: Integrating PEG-encapsulated spheroid aggregates with perfusable microvasculature provides a valuable tool for systematic engineering of the microenvironmental biophysical and biomolecular cues that influence sprouting angiogenesis. One area of ongoing exploration lies in applying this microphysiological system to elucidate the functional role of macrophages in angiogenesis, which has not yet been thoroughly investigated with *in vitro* models involving perfusable microvasculature.

Presentation: Poster

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Rapid 3D-bioprinting of a microfluidic tissue model of glioblastoma

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In the past 20 years, the combined use of soft-lithography and PDMS resulted in an enabling microfabrication strategy that has allowed a growing number of researchers to generate a variety of microfluidic models with demonstrated tissue and organ-level function, including prediction of drug-induced toxicity that is not always possible to observe in animal models (Fabre et al., 2014). Organs-on-Chips (OOCs) and Microphysiological systems (MPSs) represent a promising solution for gradually reducing, refining, and eventually replacing traditional animal testing. However, previous models suffer from a few drawbacks associated with conventional microfabrication methods and materials (Allwardt et al., 2020). In this direction, bioprinting technology could automate the fabrication processes and potentially address the throughput and reproducibility issues faced by traditional OOCs (Yu and Choudhury, 2019).

In this talk, I will present our laboratory's recent research efforts in developing a hybrid 3D-bioprinting approach that combines Stereolithography (SLA) and Extrusion bioprinting for rapid prototyping of living MPSs incorporating multiple human cell-types into a microfluidic hydrogel. Such functional scaffolds allow us to investigate cell-cell and cell-microenvironment interactions in response to fluid-flow and clinically relevant drugs. Differently than PDMS, the microfluidic hydrogel provides cells with a natural 3D space where cells can remodel the surrounding environment and physically migrate and reposition in all the three spatial dimensions to assume their native configuration and recreate higher-order tissue-structures. I will showcase our 3D-bioprinted model of glioblastoma for testing chemotherapeutics for brain cancer. This technology affords the ability to scale the number of treatment strategies tested, allows monitoring of the drug effects on normal tissues in addition to the anticancer effects, and supports the development of novel therapies against microenvironmental targets.

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Presentation: Oral

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Engineering next generation organoids with automated lab workflows

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Three-dimensional organoids derived from human induced pluripotent stem cell hold great promise to improve understanding of human biology and disease, transform drug development, drive precision medicine, and ultimately, develop transplantation-based therapies for end-stage diseases. Their ability to recapitulate facets of normal human development during *in vitro* morphogenesis produces tissue structures that re-create the architecture and physiology of human organs in remarkable detail (Takebe, 2019).

Despite considerable success in establishment of organoids representing wide range of organs in a dish, challenges remain to realize their full potential and to achieve real-life applications (Takebe, 2018). Current organoid derivation protocols, while useful for many applications, have relevant technical and conceptual limitations. Oftentimes they rely on the stochastic nature of *in vitro* self-organization and cell fate choices resulting in high line-to-line, batch-to-batch and well-to-well variability hampering the translatability of organoid systems. Moreover, the reductionist approach of organoid culture limits the full potential of this method as organoids lack more complex interactions with non-parenchymal cells such as immune cells, stroma and mechanical forces that are present in the native tissue microenvironment.

At the Center for Stem Cell and Organoid Medicine (CuSTOM) at Cincinnati Children's Hospital Medical Center we have engineered next generation organoids with cellular complexity, mechano-physiological parameters and higher-order functions similar to native tissues (e.g., innervated intestinal organoids experiencing peristaltic contractions), as well connectivity with other organs (e.g., hepato-biliary-pancreatic multi-organoid system) (Workman, 2017; Koike, 2019). In addition, we have implemented workcell automation and high content imaging to fulfill a need to standardize experimental techniques, provide scalability, improve robustness of organoid protocols, and allow *in situ* characterization of the manufactured cell products. The resulting fully automated, HTS-compatible organoid-based platforms can be used to evaluate drug effects at single organoid level via high content imaging and 3D analysis as well as multiplexed assessment of soluble biomarkers indicative of organoid health and function. All together, these new advancements bring us one step closer to realize the full potential of organoids in drug development, personalized and regenerative medicine.

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Presentation: Oral

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High-throughput nanoIEA-based assay for accessing endothelial barrier function

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Physiologically relevant vascular cell culture models require fluid flow through the endothelial lumen to achieve the highly aligned endothelial cells, which could be achieved in a live animal or vascular channel based microfluidic device connected to a syringe pump. However, those models are difficult to provide a high-throughput platform, limiting rapid screening of target molecules and therapeutics. To address this issue, we developed a nanopatterned inter-digited electrode array (nanoIEA) for high-throughput vascular research, providing highly aligned endothelial cells to recapitulate matured and intact vascular barriers in a 96-well format. The proposed nanoIEA assay provided physiologically relevant experimental models of endothelial barriers that vary in base phenotype and ensure consistency across multiple studies at the lower frequency range. Cell shape analysis, after 48 hours of seeding on nanoIEA, showed that ~80% of cells are aligned with ± 15 degrees relative to each other. To enhance the sensitivity of nanoIEA, poly(3,4-ethylenedioxythiophene) (PEDOT) solution was introduced with varying mixing volumes from 1:14 to 1:2. Under increasing PEDOT solution volume, the nanopattern depth fidelity decreased due to the hydrophilicity of the PEDOT solution. The total impedance behavior showed a faster ion transfer rate by two folds at each doubling volume of PEDOT, as assessed by electrochemical impedance spectroscopy. The impedance behavior of PEDOT-nanoIEA matched with Ran-

dles Cells equivalent circuit model. Collectively, the presented PEDOT-nanoIEA enables high-throughput, real-time analysis of endothelial cell barrier properties at low-frequency spectrum and the electrical capacitance of aligned endothelial cells at high-frequency spectrum. Quantitative impedance analysis to investigate the effect of GSK3 inhibitor on the integrity of cell-cell junction dynamics was demonstrated. Increased protein expression of ZO-1, VE-cadherin, F-actin and Claudin-5 in hCMECs via immunocytochemistry and western blot correlated with the increased impedance at both high and low spectrum range on the nanoIEA.

Presentation: Poster

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Optimization of patient-derived cell-based lung-on-a-chip using commercialized materials and devices for acute lung injury modeling

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Severe acute respiratory failure, especially emerging from new respiratory infections such as SARS, influenza, and MERS-CoV, pathophysiologically begins with acute lung injury. We developed a bioinspired lung-on-a-chip using acute lung injury patient-derived cultured cells based on commercialized materials and devices. After establishing a microenvironment simulating dynamic lung respiration, we attempted various ways to produce lung-on-a-chip proceeding by microfluidic culture. The variables include culture methods, liquid-liquid or air-liquid interface (ALI) culture, with or without mimicking respiration; pore size of the extracellular matrix coated membrane (0.45, 3, or 8 μm); order of culturing epithelial and endothelial cells, flow rate(2~5 μL/min), and so on. The two chips (Calu3-ALI-Respiration, Calu3-ALI-LPS) were solely co-cultured with pulmonary epithelial and endothelial cells, each forming an independent layer. As a result of cytokines ELISA performed, Calu3-ALI-LPS showed a significant increase in IL-6 when LPS stimulation was given compared to other control experimental conditions. The result confirms the implementation of an acute lung injury model showing IL-6 elevation by LPS induction. Our study is expected to pioneer precision medicine by cultivating human-derived cells in organ-on-chips, and open up possibilities to explore new drug candidates for acute lung injury before clinical trials.

Presentation: Poster

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Immune organoid technologies for investigating human vaccine and immunotherapy responses

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Many therapeutics currently under investigation for protecting against infectious diseases, treating autoimmunity, and eliminating tumors leverage and manipulate the immune system to elicit protection or cure disease. However, predicting the safety and efficacy of novel immunotherapies in humans is challenging because preclinical studies are largely conducted using animal or *in vitro* models that are unable to accurately reflect the complexity of human immunity. To circumvent these limitations, we have developed an *in vitro* patient-derived immune organoid platform using lymphoid tissues (e.g., tonsil, lymph node). The immune organoids can capture the original cell composition and the full functional capabilities (coordinated B and T cell responses, affinity maturation) of donor tissues. We are using this system to investigate two main areas: (1) the mechanisms of influenza vaccine efficacy in the context of how different modalities elicit distinct immune responses, which will guide future vaccine development and design, and (2) how disease-intrinsic and patient-specific factors can predict patient response following immunotherapy in lymphoma. Our current and future goals include further elucidating the intricacies of human immune responses in both health and disease to better predict patient-specific responses to immune interventions and further the global need for precision medicine.

Presentation: Poster

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Gut-on-chip for facile visualization of real-time interactions with the mucosal interface

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In the past decade, gut-on-chip systems have attracted much attention and offered promise for enhanced insight into links between intestinal and systemic health. However, most of gut-on-chip models have a horizontal epithelial orientation that makes it challenging to image the mucosal interface, including spatial distribution of bacteria with respect to the epithelium and mucus during culture. This study aims to develop and evaluate a new mesofluidic gut on chip design with a primary human intestinal epithelial monolayer cultured on a vertical hydrogel gel surface created via phase guides and the meniscus pinning effect and separating central "lumen" and side "circulatory" channels. Hydrogels of varying composition were screened for the ability to enable vertical gel wall formation with sufficient mechanical integrity to support pump-driven flow past the wall surface, and also support growth of primary organoid-derived human epithelium and stromal cells. In this study, three types of hydrogels were compared: 5% w/v Polyethylene glycol vinyl sulfone (PEG-VS) gel mixed with adhesion peptides at the 50% crosslinking ratio; EDC/NHS cross-linked type I collagen (2 mg/mL); and PEG-PLL gel made by 8.2% w/v PEG succinimidyl glutarate (PEG-SG) and 1.8% w/v Poly-L-Lysine (PLL) in cell culture media. PHISCs were seeded on the gel surface in the "lumen" channel of the chip and cultivated for 7-8 days either statically or under flow. PEG-PLL supported duodenal epithelial cell adhesion and was stable during the culture under flow, which significantly impacted the morphology of the epithelium. Collagen gels supported cell adhesion but mechanically deformed during culture, especially under flow. The ability to visualize real-time interactions of lumen contents with the mucosal interface, including bacteria (*E. coli* and *L. rhamnosus*) interaction with and penetration through the mucus overlying the epithelium, was demonstrated.

Presentation: Poster

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Recapitulating human biology, disease states, and therapeutic responses *in vitro*

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Failure of animal models to predict therapeutic responses in humans is a major problem that also brings into question their use for basic research. In this presentation, I will describe Organ-on-a-chip (Organ Chip) microfluidic devices lined with living human tissues that form tissue-tissue interfaces, reconstitute vascular perfusion and organotypic mechanical cues, integrate immune cells, contain living microbiome, and recapitulate organ-level physiology and pathophysiology with high fidelity. Work will be presented describing how single human Organ Chips and multi-organ human Body-on-Chips systems have been used to model complex diseases and rare genetic disorders, study host-microbiome interactions, quantitatively predict drug pharmacokinetic and pharmacodynamic parameters, recapitulate whole body inter-organ physiology, and reproduce human clinical responses to drugs, radiation, toxins, and infectious pathogens. My message is that the possibility that human Organ Chips can be used in lieu of animal models for drug development and as living avatars for personalized medicine is coming ever closer to becoming a reality.

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A novel immunocompetent MPS platform for modeling the cross-talk between 3D tumor tissues and circulating immune cells

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One of the most unmet challenges of microphysiological systems (MPS) is related to more predictive and fully humanized *in vitro* models for immune-oncology. The preclinical assessment of immunotherapies is currently carried out through 2D cell culture in static conditions, and *in vivo* xenografts or genetically engineered animal models, generated by the engraftment of PDXs into immunodeficient mice bearing human immune cells, but cost, time, and complete immune-compatibility remain important challenges.

A novel Multi-In Vitro Organ (MIVO) organ on a chip (OOC) platform has been recently developed to culture immunocompetent tumor models, with circulating immune cells under proper physiological culture conditions. Biologically relevant cancer samples (up to 5 mm) or patient biopsies are cultured within the MIVO chamber, while human immune cells (e.g., Natural Killer cells, NK) are able to (i) circulate in the OOC mimicking the blood capillary flow, (ii) extravasate through a permeable barrier resembling the vascular barrier, (iii) infiltrate the cancer tissue.

A human 3D neuroblastoma model with proper immunophenotype was optimized to develop a complex tumor/immune cell coculture as a paradigm of an immune-oncology screening platform (Marrella et al., 2019). NK cells have been introduced within the capillary fluid flow circulation of the MPS and their migration and infiltration towards the 3D tumor model was analyzed. Preliminary flow cytometry analysis highlighted that tumor cell viability seems to be reduced in the embedded coculture of tumor and NK cells, suggesting an effective anti-tumor NK cell-mediated activity. Importantly, a tumor-specific NK cell extravasation was observed, with DNAM1 + NK cells infiltrated within 3D tumor models.

In conclusion, we generated a functional and relevant human model, through the adoption of OOC device, that can be efficiently employed as an immune-oncology screening platform, both for pharmacological treatments and for cell-based therapies that could be "infused" into the platform for testing their journey and activity toward tumor cells.

Presentation: Oral

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Human blood-brain barrier model empowered by an engineered basement membrane

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Recent reports demonstrate that the basement membrane (BM) of the blood-brain barrier (BBB), a thin extracellular protein (ECM) sheet underneath the brain microvascular endothelial cells (BMECs), plays a vital role in maintaining brain homeostasis. However, because of the difficulty in replicating the unique properties of BM *in vitro*, most current BBB models based on Transwell or microfluidic systems employ synthetic polymer-based porous membranes to compartmentalize the luminal and abluminal side of BBB, despite their poor physiological relevance. Here, I will discuss how an engineered BM-mimicking hydrogel membrane system enables the recapitulation of human-relevant physiological BBB functions in an *in vitro* BBB model. A novel ECM hydrogel-based engineered basement membrane, named nEBM, which is supported by an electrospun nanofiber skeleton provides *in vivo* BM-like biophysical and biochemical cues to BMECs inducing high barrier function and improved efflux pump activity. Furthermore, cellular responses to stroke-like conditions are successfully recapitulated in our BBB model. Our proposed model represents a versatile tool for studying drug transport and cell transmigration across the BBB, and for developing therapeutic strategies for brain diseases.

Presentation: Oral

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ESTP/STP collaboration on complex *in vitro* models & pathology

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The European Society of Toxicologic Pathology has undertaken an effort to establish subgroups focused on new technologies that pathologists are supporting and providing expertise for. One subgroup drives expertise in Complex In Vitro Models (CIVM) & Pathology and is a collaborative effort between ESTP and the Society of Toxicologic Pathology.

The Complex In Vitro Models & Pathology working group is co-led by Nadine Stokar (Roche)/Dirk Schaudien(Fraunhofer) and Lindsay Tomlinson (Pfizer). This group is focused on pathology support for the development of CIVM including Microphysiological Systems (MPS), establishing a link to health authorities (e.g., FDA), and providing a platform for pathologists to connect with CIVM/MPS scientists and their institutions (e.g., NIH, NCATS, Fraunhofer, etc.). This working group aims to increase awareness of pathologists, investigative scientists and device engineers on the integrated role that will fuel the optimization and characterization/qualification of new complex *in vitro* models (CIVM). This includes understanding the limits/opportunities of current CIVM, physiologic endpoints that translate to *in vivo* evaluations, interpretation of readouts and synergistic communication between experts of the different disciplines. We share some examples of histological embedding techniques with further staining and digital image analysis of 3D organoids and the role that CIVM/MPS can play in toxicologic pathology safety evaluation.

Presentation: Oral

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MPSCoRe: A global working group applying open science to tackling a pandemic

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The COVID-19 pandemic has provided a clear opportunity for collaborative action between MPS model developers, pharmaceutical end users, and regulatory decision makers in creating and applying human relevant models for assessing the impact of SARs-CoV-2 on the lungs and other organ systems affected by the disease, and to develop more effective therapeutics, treatment strategies, and vaccines, whilst reducing our reliance on animal models. The response of the global MPS community has been rapid, but risks being fragmented and duplicating resource and effort. This presents significant challenges for model developers, drug/vaccine manufacturers and international regulatory authorities in coordinating efforts and understanding the utility, validity and context of use of the multitude of systems being developed. Here we describe the progress of the microphysiological systems for COVID research (MPSCoRe) working group, an international working group committed to open science approaches to support and help coordinate global MPS efforts to study COVID-19 and future infectious disease applications. A key aim of the MPSCoRe group is to connect researchers, methods developers, therapeutic/vaccine manufacturers, and regulators, to maximize the scientific, animal reduction and public health impacts that MPS offer in better understanding and treating COVID-19. We have supported expansion of the Microphysiology Systems Database (MPS-Db) to include a COVID-19 disease portal (https://mps.csb.pitt.edu/diseases/) for researchers to share data, analytical tools, model design to accelerate the adoption of MPS for disease research and therapeutics development, and the portal is currently under expansion to include an iPSC database and repository. Additional focal areas are addressing key challenges identified via a survey of the MPSCoRe working group membership. Through the MPSCoRe working group, many opportunities for cross-disciplinary coordination and collaboration are being established that will enable the scientific, human health and animal welfare impacts MPS offer to be fully realized.

Presentation: Oral

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Design and application of an adept aerosol lung-on-chip and aerosol/vapor delivery systems

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Organ-on-a-chip technology and other micro-physiological systems (MPS) were designed to recreate living tissues or organ microenvironments through precise control of the cells, extracellular matrix, and other micro-environmental factors to investigate physiological or pathological mechanisms. 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 administer 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. There have been numerous mechanisms to create aerosols, however a true commercially available aerosol delivery system has not been successfully executed in organs-on-chips or other MPS. We combined 3D printing technology with microfluidic organ-chip engineering to build a customizable open-top lung-chip specific for the evaluation of aerosol toxicity and efficacy testing. In addition to utilizing the 3D printing technology to design novel lung-chips we also used this technology to design an aerosol/vapor delivery chamber specific to the open-top lung-chips. The 3D printing provides customizable, time and cost-effective parts which allowed us to apply this technology to manufacture novel aerosol and vapor delivery systems for lung tissue exposures *in vitro*. By producing an aerosol delivery system amenable to a lung-chip, we filled a gap in our current technology by enhancing and expanding our repertoire of testing available to lung microenvironments. We designed, generated, and evaluated novel open-top lung-chips that will be used downstream to facilitate the unique needs of the U.S. Army's aerosol and vapor toxicological agent assessments. This expanded our capabilities for elucidating novel mechanisms of action, furthering our understanding of affected pathways, and identifying potential targets for the future development of diagnostics, therapeutics, and medical countermeasures.

Presentation: Poster

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Neurovascular organ chips

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The neurovascular unit (NVU) is the most restrictive barrier of the human body, essential for the function and health of the central nervous system (CNS). Despite being of major importance for evaluating brain targeting of drugs and disease-induced alternations, the established *in vitro* models of the NVU are disappointingly non-predictive. Animal *in vivo* models typically also fail to severe as human NVU and CNS models due to species-specific differences. We have developed a range of micro-engineering approaches to create vascular-mimicking, fluidic Organ-on-Chip models of the NVU. First, we created a 3D microfluidic NVU model to allow direct interaction between the human endothelium and perivascular cells such as astrocytes or pericytes. This configuration resulted in higher barrier function and more *in vivo* like responses to an acute inflammation compared to traditional culture. For evaluation of blood-brain biodistribution, we developed a compartmentalized NVU-on-Chip system. This system facilitated studies of drug-induced blood-brain-barrier alternations, metabolic and proteomic characterization, and studies of drug efflux and penetration of biopharmaceuticals, including antibodies, nano lipid carriers and viral vectors. To improve NVU functionalities and to enable patient-specific studies, we have now developed protocols to derive all relevant NVU cells from pluripotent stem cells for on-chip studies. We further increased the system's utility by impedance sensor integration for barrier studies with high temporal resolution. The whole system materials, chip and tubing, were also optimized on 15 neuropsychiatric drugs for minimizing passive compound losses.

Presentation: Oral

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Biofabrication of kidney proximal tubules and other organ structures in organ-on-chip devices

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Advances in the field of organ-on-chip (OOC) technology emerging at the convergence of microfabrication and tissue engineering have enabled a new generation of preclinical screening tools with high translational predictive value, reflected by the rapidly growing utilization of these models in the pharmaceutical industry. This interest highlights the importance of producing quality-controlled, standardized models that are stable and reproducible when produced at scale. Successful scaling up of OOC models from research and prototype stages to fully characterized commercial products is a critical step to gaining regulatory acceptance and remains among the principal challenges faced by the OOC industry. Nortis has made efforts to address this challenge through the biofabrication of pre-seeded microfluidic chips containing fully formed functional perfused tissue structures that are ready for experimental use upon arrival to customers. By providing quality batch-tested chips with readily established biology, this innovation takes the time consuming and delicate process of tissue engineering out of the hands of the customer and into those of our biomanufacturing experts, facilitating consistency in samples and faster turnaround of results. To demonstrate the various aspects required to scale up the production of our pre-seeded chips, the current presentation will highlight the implementation of quality-control and characterization parameters used in the production of our human kidney proximal tubule chip, Nortis' flagship product, and how these criteria are utilized in the biomanufacturing of our other tissue microenvironments. Biofabrication of reproducible pre-seeded models involves rigorous biological characterization of tissues, in addition to the matrix that surrounds them within the microfluidic device. Taken together, the production of reproducible, quality-controlled pre-seeded models will help to bridge the gap between industry and regulatory agencies, leading the way for the next generation of gold standard *in vitro* preclinical models.

Presentation: Oral

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Organ-on-a-chip models in early-stage drug discovery: A phenotypic screening exercise

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A successful drug development effort requires studying of compounds and targets in the correct disease context. Organ-on-a-Chip technology provides that context by comprehensively capturing human tissue physiology in healthy and diseased state. Thus far however, the technology was primarily positioned for preclinical studies as direct replacement of animal tests. Its potential to disrupt drug development by addressing compound and target discovery phases has been largely untapped.

Here, we describe screening of a library of 1546 protein kinase inhibitors on a perfused 3D angiogenesis model. The model comprises a perfused main blood vessel, grown against an extracellular matrix. Angiogenesis is triggered by exposure with a cocktail of angiogenic factors. Luminized microvessels sprout from the main vessel and have the tip-stalk hierarchy typical of the angiogenesis process. Automated robot handling was used for the various dispensing steps of the assay. Sprout and vessel morphology was recorded by high content imaging and analyzed utilizing machine learning algorithms. The assay was shown to have a robust z' factor and yielded a range of hits that were subsequently confirmed in dose response studies. The study demonstrates that Organ-on-a-Chip systems can be utilized in early stage drug screening and target discovery efforts.

Presentation: Oral

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Organs-on-chip towards fit-for-use

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In the last 10 years, organs-on-chips have steadily made it to the drug development ecosystem, being gradually adopted for efficiency and toxicity testing by the pharmaceutical, chemical, and consumer goods industry. Organs-on-chips have demonstrated their predictive accuracy simulating human responses, standing their ground as a solid alternative to animal experiments.

Currently, the main area of growth for organs-on-chips revolves around their qualification and validation for pre-clinical decision making and *in vitro* to *in vivo* translatability. In the last years, substantial data were generated by developers, and academic or industrial end-users demonstrating the fit-for-purpose of organs-on-chips. Now, ease of use, standardization, throughput, automation, and robustness arise as main technical challenges and open the question − are organs-on-chips already fit enough for standardized use?

We want to introduce how our ^{AX}Organ-on-chip technology is currently implemented in drug development and in consumer goods risk assessment, aiming to close the bench-to-bedside translational gap. The two-compartment chip enables co-culture and air-liquid interface conditions. The open-access design facilitates multiple test sampling for various downstream analyses and single-cell sequencing experiments. The technology allows to measure clinically relevant readouts and end-points, such as *in vitro* barrier integrity as an indicator of *in vivo* pulmonary edema or vascular leak, or using proteomics from cell supernatant to investigate the expression of clinical biomarkers. The ^{AX}Lung-on-chip system mimics the breathing distal airways and fosters *in vivo*-like phenotype target expression, which is essential to develop prognostic efficacy or disease models, such as pulmonary fibrosis or sepsis-induced respiratory distress syndrome. By including immunocompetent cells, safety testing of new immunotherapies demonstrated predictive power for lung toxicity studies.

In summary, we aim to give a snapshot of how ^{AX}Organ-on-chips are adaptable for a wide range of biomodels and appliances. By introducing case studies, we are demonstrating their fit-for-purpose and added value in the drug discovery process from the R&D to the pre-clinical and regulatory submission phase. Our next goal is to make the technology fit-for-use by increasing throughput and standardization and adapting it to automated read-out options.

Presentation: Oral

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Validation of an MPS human tumor model: Vascularized colon cancer micro-tumors recapitulate patient drug responses

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Accurately modeling tumor biology is critical both in the development of new drugs and in the selection of appropriate therapies for patients. The vascularized micro-tumor (VMT) is a physiologic preclinical cancer model that incorporates several key features of the native human tumor microenvironment in a microphysiological system platform. These features include perfused vasculature, stromal cells and a complex matrix, all within a transparent microfluidic device that allows for real-time study of drug responses and tumor-stromal interactions. Critically, all nutrients and drugs reach the tumor through living, human blood vessels. We have validated the VMT platform for the study of colorectal cancer (CRC), the second leading cause of cancer-related deaths, by showing that gene expression, tumor heterogeneity, and treatment response in the VMT more closely model CRC tumor clinicopathology than current standard drug screening modalities, including 2D monolayer culture and 3D spheroids. We have now optimized methods for generating patient-derived VMTs (pVMT) using fresh patient-derived colorectal cancer (CRC) biopsies and surgical resections. Tumor growth in the pVMT recapitulates histological features, metabolic heterogeneity and drug responses of actual CRC patient tumors. Patient responses to drug treatment were heterogeneous, with some responding clinically to standard-of-care (SOC) drugs while others progressed. Importantly this heterogeneity in response is captured by the pVMT where we also saw complete resistance to some SOC treatments. Finally, using the pVMT we have identified pathways that are critical to tumor growth in patient tumors that were resistant to SOC treatment. These studies demonstrate the potential of the VMT for improving drug discovery and patient drug selection.

Presentation: Oral

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Organoid Intelligence (O.I.): The new frontier in biocomputing and intelligence-in-a-dish

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Recent advances in human stem-cell-derived brain organoids promise to replicate critical aspects of learning and memory *in vitro*. Coining the term Organoid Intelligence (O.I.) to encompass these technical developments, we are showcasing the new multi-disciplinary scientific and engineering field of O.I. and its potential to revolutionize computing. We define O.I. as a new frontier of a biocomputing revolution. As such, we provide a vision for its development over the coming decade and highlight the important societal and ethical considerations it entails. We delineate its principles and potential benefits over computational A.I. by using biological learning to vastly improve the speed, quality and energy efficiency of computing for the benefit of science and society. Here, we describe the scientific and technological basis of O.I. – bringing to the fore the latest collaborative brain cell culture/bio-engineering advances, providing the foundation for the new O.I. paradigm (allowing mass production of standardized, 3-dimensional, myelinated brain organoids with high cell density encapsulated in to the multielectrode cages, EEG). We share a comprehensive vision of an iterative, multidisciplinary research and development trajectory that aims to further scale up the production of organoids housed in novel electrode arrays. We present the challenges and nascent solutions being developed with the potential to pioneer novel biocomputing models via stimulus-response training and organoid-computer interfaces – assessing the true learning potential of O.I. We delineate the necessary roles of the various disciplines involved in this inherently multidisciplinary new field – including electrophysiology, bioengineering, brain modelling, A.I./big data, and bioethics. O.I application goes beyond modeling of learning and memory to biological and hybrid computing as well as disease modeling.

Presentation: Oral

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Guidelines for microfluidics: How to simplify your OoC life?

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An organ-on-chip needs… a microfluidic chip! Whatever the organ, the application, the maturity, the material, it is key to follow some rules in each research and development phase. What are the main design guidelines? How to connect the chip to avoid leakages? What are the fluidic conditions for each type of cell or organ? The expanding field of the OOC offers a wide variety of innovations, designs. And it will be necessary to structure the developments, at least to be able to compare results. The microfluidic community is now organized to address these topics and to propose to the MPS community a toolbox to speed up the development of the next generation of organ on chips. Through results and examples, the presentation will illustrate the different initiatives coordinated by the MicroFluidics Association: ISO working group, MFMET project…The talk will also focus on the roadmap: current and future. And how to link the microfluidics roadmap with the MPS needs.

Presentation: Oral

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Tonsil organoids to investigate human adaptive immunity

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Influenza virus infections cause significant global morbidity and mortality and pose a serious pandemic risk. Current vaccines elicit strain-specific responses and have limited efficacy. There is an urgent need for a broadly protective influenza vaccine strategy. Designing such a vaccine will require a comprehensive understanding of how features from both the host and the antigen modulate the magnitude, quality, and breadth of the influenza-specific response. Most human influenza studies have been limited to peripheral blood sampling, even though the critical cellular decisions that lead to productive adaptive immunity occur within lymphoid tissues. Our goal is to define the dynamics of the lymphoid tissue microenvironment, including cell-cell interactions and signaling pathways that elicit protective immune responses in humans. To address this question, we use a high throughput *in vitro* organoid platform derived from primary human tonsil tissues. Tonsils are an accessible lymphoid and mucosal tissue that can be collected from otherwise-healthy patients undergoing tonsillectomy for hypertrophy or obstructive sleep apnea. Immune organoids derived from tonsils can be used to accurately model human germinal center responses, specific antibody secretion, and T cell activation in response to influenza antigens. They are also able to capture host-mediated inter-individual immune variation related to patient age, sex, and immune history. Furthermore, tonsil organoids can be used to track the kinetics of the adaptive immune response and enable the mechanistic insights that will be required to rationally design vaccine candidates.

Presentation: Oral

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Effect of perfusion on human proximal tubule kidney cells in culture: Applications in toxicology

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Introduction: The proximal tubule of the kidney consists of epithelial cells (RPTEC) that are involved in secretion and reabsorption of molecules, drugs, and metabolites via active transporters. These cells are common targets for toxicity. RPTECs form a tight barrier and are ciliated and constantly exposed to flow. Vitrofluid is a multicompartmental microphysiological system for perfused cultures and can accommodate one or several tissues. Here, we set off to characterize RPTECs maintained under static or dynamic culture conditions in terms of cell behavior and response to toxicants.

Results: The human kidney barrier model we developed with human primary RPTECs under both static and dynamic conditions was viable and functional for 14 days. The cells formed an integral barrier characterized by tight junctions and low permeability. The RPTEC barrier retained the ability to reabsorb albumin and expressed several proximal tubule transporters (SLC22a2, SLC22a6, CUBN, and AQP1) but did not express LRP2 or hOAT1. Shear stress did not have any damaging effects, as assessed by barrier integrity and expression of transporters. However, continuous medium flow led to a marked change in the morphology of the cilia, which became significantly longer and aligned with the flow. RPTECs in static conditions responded to most test compounds with an expected decrease in cell viability. As an exception, tenofovir did not cause cytotoxicity, as it requires uptake via hOAT-1. Furthermore, studies with colistin under static or flow conditions showed that RPTECs grown in both systems exhibited a similar dose-response behavior to the compound.

Conclusions: Our results demonstrate the suitability of a human RPTEC model for transport and nephrotoxicity studies. Physiological flow conditions changed the morphology of the cilia without affecting barrier function or the sensitivity of the cells to colistin. Further studies should evaluate the sensitivity of the cells under flow conditions to other compounds. In addition, exposure of proximal tubular cells to more physiological conditions (such as artificial urine) on their basolateral/luminal side could help restore LRP2 and hOAT1 expression.

Presentation: Poster

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Standards supporting innovation: The case of organ-on-chip

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Standards are widely used by companies to support commercial activities on mature technologies and products and are very useful upstream in transferring technologies from R&D into applications and commerce. In a scientific environment, standards can also be of value, together with publications and patents, to advance the state of the art in many research fields.

In the life science domain however, the lack of standardization roadmaps and strategies often means that standards play a minor role. To address this, many consortia have been working to promote the development and uptake of standards, especially for Organ-on-Chip (OoC) technologies. There is a strong justification for translating scientific evidence into standards, supporting the advancement of the OoC field towards wide acceptance by end-users, creating a robust and vibrant marketplace for human-relevant alternatives to animal testing.

By describing specific requirements and performance of elements in an open, clear and structured way, standards can help overcome technical and biomedical challenges, reach consensus for terminology, establish experimental and reporting methods, and set a frame for benchmarking. End-users in industry and academia are asking for simple to use, cost effective OoC devices that can be purchased off the shelf and then adapted to specific applications. To fully trust these products, characterization of the technological components, interoperability among different designs and compatibility with other laboratory equipment needs to be ensured. To reach regulatory acceptance in the chemicals and pharmaceutical domains, standards can be used to consistently qualify OoC devices, demonstrating their scientific reliability and relevance, thus giving confidence that the data that OoC produce can be used to ensure solid, science-based decisions to protect human health and the environment.

To encourage the development and use of standards, it is now necessary to involve Standards Development Organizations as key actors in the formal standardization process. As a concrete action, CEN-CENELEC has recently established the OoC Focus Group as a European coordination platform. The Focus Group will define a roadmap and will stimulate and coordinate standardization efforts relevant to the field of OoC, setting priorities and identifying the Technical Committees to be involved for formal standards development and approval.

Presentation: Oral

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A human choroid plexus-on-a-chip to study the effect of hydrocephalus-associated inflammation on cerebrospinal fluid secretion

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Hydrocephalus is a neurological condition characterized by the buildup of cerebrospinal fluid (CSF) in the ventricles of the brain, which can disrupt brain development, lead to cognitive, motor and sensory function deficits, and if untreated, lead to brain herniation and death. Presently, no successful non-surgical intervention strategies exist because pharmaceutical CSF regulation demands a mechanistic understanding of CSF secretion through the choroid plexus (CP). Conventional animal models used to study hydrocephalus are expensive, introduce numerous confounding variables, limit access to the CP, and are plagued by variability. Existing *in vitro* models such as Ussing chambers and transwell plates lack physiologic complexity. There is a critical need for an *in vitro* platform that will bridge the gap between these models and allow us to study CSF secretion at the CP in the context of hydrocephalus.

To directly address this need we developed an organ-on-a-chip model of the CP. Using computational fluid dynamics models we ascertained the ideal dimensions that would allow us to mimic the physiological shear in the luminal and abluminal compartments of a two channel microfluidic device. Next, we applied 3D printing and soft lithography to fabricate the device and sandwiched a polyethylene terephthalate (PET) membrane between the two channels. Immunofluorescent labeling confirmed that CP epithelial cells grown on the PET membrane inside the abluminal channel established a confluent monolayer, oriented themselves correctly and expressed critical tight junction components. Currently we are in the process of using fluorescently labelled dextran and electrodes incorporated into the chip to track changes in the barrier function of the monolayer. Future work involves testing our hypothesis regarding inflammation mediated barrier integrity loss and subsequent CSF hypersecretion at the CP as it pertains to hydrocephalus. The human CP-on-a-chip has the potential to replace currently used pre-clinical animal studies with human-relevant systems in translational research and reveal previously undiscovered transport mechanisms at the CP.

Presentation: Poster

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Modeling Shiga toxin induced renal microvascular injury and thrombotic microangiopathy with a flow-directed kidney vascular microphysiologic system

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Background: Hemolytic uremic syndrome (HUS) results in acute kidney failure, and is caused by kidney endothelial injury that leads to hemolytic anemia, thrombocytopenia and thrombotic microangiopathy. HUS in children is most frequently due to Shiga toxin E. Coli-HUS (STEC-HUS). In STEC-HUS, bacterially produced Shiga toxin (Stx) in the intestine translocates to the bloodstream, and preferentially binds to the glycosphingolipid globotriaosylceramide (Gb3) which is robustly expressed on the surface of kidney microvascular endothelium. Following this cell surface binding, Stx-Gb3 is internalized and exerts multiple cytotoxic effects leading to kidney endothelial injury and death. No targeted therapies for Shiga toxin E. Coli-HUS (STEC-HUS) exist, but glucosylceramide synthase inhibitors (GCSi) are orally available, FDA-approved medications targeting glycosphingolipid synthetic pathways, offering an intriguing drug-repurposing strategy for preventing STEC-HUS. Our human kidney microvascular cells (HKMECs) and microphysiologic vessel system provides ideal platforms to investigate Stx-mediated kidney microvascular injury and blood-endothelial interaction, permitting pre-clinical evaluation of GCSi in preventing Stx-associated toxicity.

Objectives: (1) Characterize the responses of HKMECs to Stx in 2D monolayer and 3D perfusable microvessel platforms under flow. (2) Assess the efficacy of GCSi in preventing Stx binding, injury, and associated toxicity.

Design/methods: HKMECs were treated with Stx1 or Stx2 in tissue culture and 3D microvessel platforms, evaluating for cell death, cell injury, and morphology in the absence and presence of GCSi. Treated microvessels were also perfused with human blood to assess endothelial activation and blood-endothelial interactions.

Results: HKMECs demonstrated significant cell-surface Gb3 expression, in contrast to other endothelial cell types such as HUVECs and HCAECs. Immortalized HKMECs were generated, showing consistent Gb3 expression, and Stx injury as seen with primary HKMECs. Stx binding led to a dose-dependent cell death in HKMECs, with significant injury and blood-endothelial interactions occurring in engineered microvessels. These effects were reversed with GCSi treatment.

Conclusions: Stx treatment of HKMECs demonstrate robust binding, internalization, and injury consistent with clinical STEC-HUS, and is completely blocked by GCSi in both culture and microvascular models. These results provide strong pre-clinical evidence for further investigation into the use of these GCSi in Stx-HUS.

Presentation: Poster

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Identifying therapeutic targets for CsA mediated kidney microvascular injuries

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The kidney microvasculature consists of highly specialized endothelia, with distinct functional, transcriptional, and morphologic characteristics that allow for the kidney to serve a principal regulator of homeostasis. Immunosuppressive agents such as calcineurin inhibitors (CNI) have provided significant improvements in tolerance of kidney and other solid organ transplants, but remain a concern for nephrotoxicity due to their toxic effects and relatively narrow therapeutic window. CNIs are causative for both acute and chronic microvascular injury, and these side-effects limit dosing and complicate adequate immunosuppression in the balance between tolerance and preservation of graft function. Patterns of injury to the endothelium have been investigated, but a better understanding of the underlying mechanistic pathways will provide additional insight into pathogenesis and tailored immunosuppressive regimens.

Using engineered models of kidney microvasculature, we have recently shown that CNIs impact the structure and function of endothelial cell fenestrae under flow with impairment of NFAT1-VEGF signaling. We have further screened 38 kinase inhibitors with or without CsA treatment and utilized kinase inhibitor regression analysis to identify kinases that are protective and disruptive to these human kidney microvascular endothelial cells. Moreover, the potential drugs are predicted to optimize the protection, which provide potential therapeutic candidates to limit vascular injuries during CNI administration.

Presentation: Poster

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From Good Cell and Tissue Culture Practice (GCCP 2.0) to Good *In Vitro* Reporting Standards (GIVReSt)

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A number of cell culture technologies have become more broadly available, which allow overcoming shortcomings of traditional culture. These include the use of stem-cell derived cells, cocultures of different cell types, scaffolds and extracellular matrices, perfusion, 3D culture, tissue architecture and organ functionality. The physiological relevance is further enhanced by the measurement of biomarkers and more wholesome assessment cell responses by high-content methods. These approaches are still rarely combined to create MPS and we do not argue that all cell culture needs to be that sophisticated. The efforts are determined by the test purposes. If only a very specific molecular target to cell response is of interest, a very simple model, which reflects this might be suited to allow standardization and high-throughput. However, the less defined the endpoint of interest and cellular response are, the better we should approximate organ- or tissue-like culture conditions to make physiological responses more probable. Important progress in the quality assurance and reporting on cell cultures as well as the validation of cellular test systems bring the utility of cell cultures to a new level. In the safety sciences especially this is a major prerequisite for meaningful and reliable results, ultimately supporting risk assessment and product development decisions. Other area of the life sciences and decision-taking in product development similarly profit from quality assurance of these tools.

Regulatory toxicology has only slowly begun to embrace these new approaches. However, major parts of toxicology have not yet found *in vitro* solutions. The lecture summarizes the lessons learned from the development, validation and acceptance of alternative methods for the creation of quality assurance of MPS. We most recently drafted Good Cell and Tissue Culture Practice (GCCP) 2.0, which expands the original GCCP guidance from 2005 to the new approaches, and Good In Vitro Reporting Standards (GIVReSt) under development support their implementation.

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Instrumented microphysiological analytic platforms for precision measurement and manipulation of tissue functions

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My laboratory research focuses on the mechanobiology of human diseases and the development of human organ/tissue-on-a-chip platform technologies for disease modeling, drug screening, and precision medicine. In this talk, I will introduce instrumented microphysiological systems platforms integrated with biosensors and/or actuators developed in our laboratory, including high-throughput microphysiological model of muscular dystrophy, micro/nano-fabricated platforms for drug-induced cardiotoxicity, and microfluidic blood-brain barrier model for drug delivery studies. Using our multi-scale biofabrication techniques combined with functional matrices, I will highlight how our biomimetic tissue chip models helps to gain a better understanding of the structure-function relationship in complex 3D tissues, and serve as emerging platforms for disease biology studies and biotherapeutic development.

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Standards for integrating heterogenous data and metadata from organ-on-chip technologies

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The increasing flood and complexity of data in life sciences, especially in interdisciplinary fields such as Organ-on-Chip that bundles data from different sources and technologies, calls for standardization of data and of its documentation to render the data "FAIR" (Findable, Accessible, Interoperable and Reusable). This comprises not only structured data formats, but also standards for metadata (data describing the data and its context), such as the description of applied biological material, methods, and workflows for processing, storage, exchange, integration or analysis of the data, as well as of derived computational models. Hence, standards for formatting and describing data, workflows and computer models have become important, especially for data integration across the manifold methods applied for Organ-on-Chip, or across the biological scales for multiscale approaches.

To this end many grassroots standards for the different types of data, models and their metadata have been defined by the scientific communities and are driven by standardization initiatives such as for example COMBINE (http://co.mbine.org) for computational modelling approaches or EU-STANDS4PM (https://www.eu-stands4pm.eu) for *in silico* methods in personalized medicine. For facilitating the integration of data in the interdisciplinary field of Organ-on-Chip, (meta-)data standards have to be harmonized also across technological silos to become interoperable and allow interfacing between the often heterogenous datasets and their manifold metadata. To support this, standards are defined by the International Organization for Standardization (ISO) in its technical committee ISO/TC 276 – Biotechnology (https://www.iso.org/committee/4514241.html), such as the emerging standard ISO 20691 "Requirements for data formatting and description in the life sciences" (https://www.iso.org/standard/68848.html) that defines a framework and guideline for interoperable data standards in the life sciences and their application in different domains. Moreover, data provenance standards like ISO 23494 (https://www.iso.org/standard/80715.html) are currently developed, that will allow to trace back data in the chain of data or sample processing back to its origin for a better transparency of the processes. Such standards aim at enhancing the interoperability of data and therefore facilitate complex and multiscale data bundling and integration.

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Biophysics-based computational modelling as a tool for translational research and drug screening in microphysiological system

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Introduction: The recent developments in the *in vitro* technology of human induced pluripotent stem cells (hiPSC) represent a key component in success of the microphysiological systems (MSPs). hiPSCs represent a virtually infinite pool of human-based cells. Unfortunately, the costs of large-scale *in vitro* tests and the time they require can represent a bottleneck. Exploiting computational modeling and simulation (CM&S) can overcome this limitation, as has been shown for hiPSC-derived cardiomyocytes (hiPSC-CMs) and primary human ventricular CMs (hV-CMs) in cardiac safety and efficacy studies. Here, we present our recent efforts to use CM&S in translational research and drug screening and highlight their crucial contribution to MPSs and their development.

Approach: We have developed physiology-based computational cell models that not only incorporate the standard mathematical formulations of transmembrane ionic currents and dynamics of intracellular ionic concentrations, but also account for characteristic spontaneous activation and immature (ultra-)structural features of hiPSC-CM. Furthermore, we have implemented *in silico* populations of hiPSC-CMs that replicate the huge variability observed *in vitro*, and recently also incorporating actin and myosin interactions, which enables us to go beyond electrophysiology and simulate contraction as well. In all these models, the system parameters reflect the biophysical counterparts, thus recapitulating the cellular basis for ionic handling and contractile machinery.

Discussion: Our simulation results show that, while hiPSC-CMs recapitulate many physiological properties of hV-CMs, they have 1) a weaker repolarization reserve, 2) more dramatic disease phenotypes due to less robustness, and 3) they can show different responses to specific drugs, due to the diverse expression of few ion currents than in hV-CMs.

The immature phenotype does not lessen hiPSC-CMs' value – studied *in vitro* or *in silico* – for drug tests as "the most human *in vitro* model available", capable of obtaining better specificity and comparable sensitivity in comparison to *in vivo* and *ex vivo* animal studies [8]. Physiology-based CM&S provides an important parallel strategy for overcoming some of the limitations of experimental MPS models. The combination has potential in ensuring better translation of the *in vitro* results and, for example, speeding up pipeline for prescreening of potential pharmacological compounds.

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Development of image-based analysis to support high throughput screening using complex biology on MPS

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The key breakthrough of MPS is providing a platform simulating organs, tissues, or recapitulating diseases *in vivo* in an effort of overcoming challenges from studies using physiologically irrelevant 2D monocultures. Many MPS platforms support the co-culturing of epithelial cells, fibroblast, and endothelial cells. Immunofluorescence using specific antibodies staining for each cell type in conjunction with the use of high throughput microscopy or high content imaging enables us to discern phenotypic changes in cell populations as endpoints in MPS. Due to the complexity of the co-culturing different types of cells, the challenges in the data analysis may be to identify subtle changes in cellular morphology in each population and objectively determine the region of interest (ROI), and how to handle big data sets. In an attempt to use MPS for High Throughput Screening (HTS), the data analysis should be robust, and reproducible. Qureator is developing image-based data analysis tools based on a public platform, Open Microscopy Environment (OMERO) that can contribute to the standardization and harmonization of complex data analysis using MPS. We will discuss the Age-related Macular Degeneration (AMD) model on our proprietary MPS, Curiochips as a case study. Of note, there is no treatment approved for the dry type of AMD. In the AMD model, the Retinal Pigment Epithelium (RPE) was co-cultured with endothelial cells. The RPE barrier was visualized by anti-F-actin and anti-Zo-1 and the 3D blood vessel network was visualized by anti-CD31 and co-culturing with other supporting cells. The functional analysis of the RPE barrier was validated by a permeability assay and Zo-1 staining for the tight junctions in OMERO. The 3D AMD model on Curiochips and the OMERO-based data analysis demonstrated the detrimental effect of damaging agents such as oxidative stress, hypoxia, and inflammatory factors and increase of VEGF that led to neovascularization. In conclusion, the AMD platform of AMD-on-a-chip and the image-based analysis tool is an efficient tool for recapitulating dry AMD and can be utilized as an HTS drug discovery platform.

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Embracing diversity in a microphysiological world

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Obesity has become a major public health crisis in the United States, with an estimated economic burden of $100 billion annually. The economic and health burden is easily tripled when factoring the increased risks of developing associated co-morbidities such as type II diabetes, cardiovascular disease, cancer and complications as result of infectious diseases, as we observed recently with Covid-19. Once associated primarily with high-income countries, obesity is now prevalent in low- and middle-income countries and is recognized by the CDC as a metabolic status that triples the risk of Covid-19-associated hospitalization. There is a need to develop platforms that provide earlier indicators of toxicity and drug efficacy using human-based systems to accelerate drug development and reduce the need for drug testing in animal models, which is time consuming, costly, and often does not predict the adverse effects in humans.

We have developed a proprietary human scaffold – Obagel™ – that, when combined with primary human stromal vascular fraction, creates 3-D tissue engineered adipose depots called "Fat-on-a-Chip," for disease modeling and drug discovery.

The molecular, functional/physiological, and cellular characterization of our "fat-on-a-chip" system demonstrates a scalable ability to manufacture these "WAT-on-a-chip" or "BAT-on-a-Chip" constructs for modeling white and brown adipose, respectively. Furthermore, we licensed the use of a matrix mimicry system for *in vitro* adipose hypertrophy, using fiber networks. These self-assembling adipose depots, maintained for extended culture periods with minimal effort, can be used to model human adipose tissue that is representative of individual donor demographics, including body mass index, age, gender, ethnicity, and metabolic disease status.

The use of "Fat-on-a-Chip" products can have a significant impact on the translation and discovery-based endpoint phases of therapeutic development, thereby minimizing the use of small animal studies required for predicting toxicity and therapeutic responses.

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Presentation: Oral

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The SAEC-PBMC co-culture: An immunocompetent human airway *in vitro* model for the safety assessment of T-cell bispecific antibodies

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In the last decades, novel therapeutics engaging the immune system in the context of cancer therapy have emerged rapidly. However, increasingly complex T-cell bispecific antibodies (TCBs) pose a major challenge for preclinical safety assessment due to limited inter-species cross-reactivity and the absence of the human immune system in classical *in vitro* assays. The target proteins are often present in healthy tissue, bearing the risk of on-target/off-tumor effects. In the past, this has led to termination of animal studies and adverse events in clinical trials.

For a better assessment of such liabilities in the lung, peripheral blood mononuclear cells (PBMCs) were integrated into an air-liquid interface (ALI) culture of primary airway epithelial cells (SAEC). ALI cultures recapitulated the diverse subsets found in the human airway including ciliated and secretory cells, providing a physiologically relevant setup for *in vitro* safety assessment. To account for donor variability, a bank of SAEC donors sourced from external providers has been composed. These donors were carefully selected based on the commercial availability of MHC-class-I-matched PBMCs. An internally developed eplet matching algorithm was used for matching of lung tissue donors and PBMC donors. The aim of HLA-matching was to minimize alloreactivity-driven overestimation of compound-induced immune-mediated toxicities. ALI cultures were established over the course of four weeks before PBMC co-culture and TCB treatment were initiated and closely monitored over three days. Cytokine release was measured daily in the culture supernatant using a 10-Plex Luminex assay. After three days of treatment, target cell killing, PBMC activation and TCB binding were assessed by flow cytometry. The model was validated with a T-cell receptor-mimic antibody, resulting in increased TCB-induced killing of SAEC, T-cell activation and pro-inflammatory cytokine release over the isotype control.

In conclusion, the SAEC-PBMC co-culture allowed us to quantify cytokine release, PBMC activation and target cell killing exerted from investigative TCBs. Integrating and interpreting such data in the context of results from known therapeutics will enable a better prediction of potential toxicological effects of novel antibody therapeutics. In the future, this approach will also help to further understand the importance of MHC-I matching for *in vitro* co-culture experiments.

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Toxicity mechanisms of novel organophosphate flame retardants: Employing adverse outcome pathways for metabolic disruption

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Organophosphate flame retardants (OPFRs) have been consistently detected in increasing concentrations in the environmental and human matrices. Growing evidence suggests that numerous health hazards are associated with exposure to OPFRs, including metabolic disease. However, the underlying mechanisms linking exposure with metabolic pathologies remain poorly understood. In the present study, we used human liver cell culture (monolayer and 3D spheroids) to characterize the toxicological effects and potential mechanisms for OPFRs-mediated metabolic disruption. We examined nine OPFRs through *in vitro* bioassays toolbox to assess the key events (KEs) such as lipid accumulation, mitochondrial dysfunction, and expression of several genes related to lipid metabolism and utilized the *in silico* approach to identify the putative molecular initiating events (MIEs). A high throughput shotgun lipidomic analysis was performed on the individual 3D hepatospheroids cell culture to explore the potential mechanisms for selected OPFR-mediated effects on intercellular lipid profiles.

Our findings suggest that OPFRs, such as tricresyl phosphate (TMPP), triphenyl phosphate (TPHP), 2-ethylhexyl diphenyl phosphate (EHDPP), and tris(1,3-dichloropropan-2-yl) phosphate (TDCIPP) induced the lipid accumulation in human liver cell culture by altering the expression of genes encoding for hepatic lipogenesis and mitochondrial dysfunction. Available data from ToxCast and *in silico* molecular docking suggested pregnane X receptor (PXR) and peroxisome proliferator-activated receptor-gamma (PPARγ) as potential MIEs. Moreover, EHDPP altered the lipidome profile of human 3D hepatospheroids and dysregulated several lipid classes, including sterol lipids, sphingolipids, glycerolipids, glycerophospholipids, and fatty acyls. Our study identifies several OPFRs as a potential risk factor for metabolic pathologies and brings novel insights into the metabolic disrupting effects of OPFRs such as EHDPP. It demonstrates the utility of an AOP-based strategy for screening, prioritizing chemicals, and elucidating the molecular mechanisms of toxicity, as well as demonstrates the utility of 3D cell culture such as hepatospheroids as an *in vitro* cell culture model complemented with omics technology (e.g., lipidomic) for high throughput toxicity studies.

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Multifaceted approach to examine the effects of mechanical forces on uterine fibroid cells

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Uterine fibroids are stiff, benign, monoclonal tumors and represent the leading cause of hysterectomy. We hypothesize that the mechanical forces that the uterine fibroid cells are exposed to throughout the menstrual cycle enhance fibroid growth. Our goal is to compare the responses of fibroid and normal myometrial cells after stimulation or inhibition of mechanosensitive pathways. Patient-matched myometrial and fibroid tissue samples were obtained at the time of myomectomy or hysterectomy. Three models were used to study the impacts of different mechanical forces at the cell and tissue level: monolayer cultures, collagen hydrogels, and a 3-dimensional (3D) spheroid model. Monolayer cells were analyzed with different substrate stiffness using Cytosoft® elastic modulus plates, with and without inhibitors SB505124 (TGF-β), Metformin (IGF-1), Ly294002 (PI3K), and G15 (GPER-1). Fibroid and myometrial cell proliferation were differentially affected by both substrate stiffness and the inhibitors of the IGF-1 and PI3K pathways. Inhibition of the PI3K pathway led to over a 20% reduction in fibroid cell number as compared to vehicle control. Contractility assays were performed; myometrial cells were more contractile than fibroid cells and inhibitors to GPER-1 and TGF-βinhibited contraction significantly, by more than 30%. The growth of fibroid and myometrial 3D spheroids were analyzed over a five day period to determine baseline growth over time. The myometrial spheroids expanded 39% and the fibroid spheroids expanded 49%. Our results demonstrate a clear difference in response of fibroid cells to mechanical forces compared to myometrial cells and demonstrate techniques that can be used to elucidate the effects of mechanical forces at the cellular and tissue level. Continuing to define the mechanotransduction processes in more detail will allow for future areas of focus for non-hormonal, fertility sparing treatment options for uterine fibroids.

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Vascular pancreatic islet (vPANIS) 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 more than 90% of cases (Sun et al., 2022). T2D development correlates with a toxic bioenvironment due to a diet high in fats and sugars and inactive lifestyle that results in the dysfunction of pancreatic islets' insulin secretion. With the development of an islet-on-chip T2D model, high throughput testing can be implemented in treatment development that can aid in expediting the transition from initial testing to clinical trials. This project aims to develop a type 2 diabetic islet-on-chip model utilizing both primary human islets and human induced pluripotent stem cell (hiPSC)-derived islet organoids.

Our vascular pancreatic islet (vPANIS) system utilizes the commercially available Organ-on-Chip device from Micronit. The system is comprised of three glass layers to form 2-chambers with dedicated inlets and outlets partitioned by a porous polyester membrane, providing a variety of possible flow configurations through the system. We developed a novel strategy of hydrogel-supported islet micropatterning on the membrane to maintain the islets' 3D spheroidal morphology. A COMSOL-based *in silico* flow field model informed this system design and was verified experimentally using *in situ* imaging of FITC labelled dextran flow.

The primary islets retained high viability (> 95%) and glucose stimulated insulin secretion (GSIS) over extended periods of 21 days (stimulation index (SI) 16 mM/3 mM glucose > 3) and 28 days (SI > 1.5). Islet phenotype was maintained, with ≈70% C-peptide cells and 21% glucagon. The progression of type 2 diabetes was modeled by simulating the diseased states of glucotoxicity, lipotoxicity, and glucolipotoxicity with long term exposure to pathological glucose and/or free fatty acid concentrations. The resulting islets showed lower C-peptide cells and loss in GSIS functionality (stimulation index ≈1). In parallel, hiPSC-derived islet organoids were developed to replace the primary islets as a regenerative cell source. As with the primary islets, the hiPSC-islet system

retained viability and showed GSIS responsiveness (SI > 1.7) over a 14-day period with the direction of simulating T2D conditions. Additionally, we coupled our vPANIS with the UPitt Drug Discovery Institute's liver MPS (vLAMPS) to explore the relationship between T2D liver and islets.

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3Rs opportunities for organ-on-a-chip technologies: An analysis of the landscape

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Organ-on-a-chip (OoC) technologies are a key area of development within the microphysiological systems (MPS) field offering significant opportunity to implement the 3Rs across the research landscape (Marx et al., 2020). They are a promising tool to reduce the reliance on animal models by providing faster, cheaper and more physiologically relevant human cell-based models for use in basic and applied research.

The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has invested significantly in the development and application of OoC technologies across multiple fields. We recently completed an analysis of the OoC landscape, using the results from a survey targeting end-users, to inform future strategy and investment (NC3R, 2021). The survey provided insights into how OoC is being used across different sectors, the 3Rs impacts and opportunities for wider adoption.

Respondents were from 11 countries and a range of sectors, with the majority (87%) either currently using or considering using OoC technology. Results indicated significant interest in OoC, particularly in the pharmaceutical and academic sectors, and an expectation that OoCs will be deployed in the future for decision making throughout the preclinical and clinical pipeline.

The results also identified barriers that, if overcome, will allow wider adoption for routine use. Over 40% of respondents not using OoC indicated that the technologies were complex to use and labor intensive, however our report identified ways in which the community is working to overcome these challenges.

Here we will present the data from the survey and recommendations describing how we will continue to support the development and application of OoC to deliver real-world 3Rs benefits across the biosciences. We will also showcase examples of MPS models developed through our CRACK IT open innovation platform which connects academics, small businesses, and large industry in developing new models to address scientific and business challenges.

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Development and characterization of a perfused blood-brain barrier model derived from hCMEC/D3 cell line

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The development of molecules for the treatment of diseases of the central nervous system (CNS) is strongly dependent on their ability to cross the blood-brain barrier (BBB), a structure that severely restricts the passage of many macromolecules to the CNS. The development of robust *in vitro* BBB models displaying physiologically relevant features is crucial to elucidate the interactions between the BBB and potential drugs. Here, we established a perfused human BBB model in a microfluidic platform, the OrganoPlate (Mimetas). In a 3-lane configuration, human immortalized endothelial cells (hCMEC/D3) were grown against a collagen type I- based extracellular matrix. A tubular structure was observed by confocal microscopy after 5 days in culture. The integrity of the barrier was confirmed by decreased paracellular permeability of fluorescent-labeled molecules (Lucifer yellow and IgG). Expression of main BBB markers, i.e., the tight junction proteins ZO1, Claudin5 and Occludin, and of key influx/efflux transporters (Glut1, transferrin receptor, P-glycoprotein and BCRP) was confirmed by immunostaining or qRT-PCR. Moreover, the functional activities of P- glycoprotein and transferrin receptor could also be demonstrated. P-glycoprotein inhibition with cyclosporine A

led to the increased cellular accumulation of its specific substrate, rhodamine 123. The cellular uptake of fluorescent-labeled transferrin could be partly blocked by competition with a 10-fold excess of unlabeled transferrin. To evaluate the influence of factors produced by pericytes, neurons and glial cells on the barrier, the endothelial cells were treated with conditioned media (from brain primary pericytes and from neurons and astrocytes differentiated from the neural progenitor cell line ReN VM).

Our results show that hCMEC/D3 monocultures display more physiological properties when cultured in a microfluidic device than when maintained in transwells in the absence of flow. Under the tested experimental conditions, factors produced by other cells of the neurovascular unit (pericytes, neurons and astrocytes) did not improve the properties of the barrier. In conclusion, monocultures of hCMEC/D3 cells under flow display physiological cell morphology, barrier tightness, and transporter function typical of the BBB. This model is suitable for the assessment of cellular uptake and transcytosis through the BBB of potential drug candidates.

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Comprehensive multiplexed superfusion system enables physiological emulation in cell culture: Exemplification by persistent circadian entrainment

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Cells and tissues are routinely cultured *in vitro* for biological research with findings being extrapolated to their host organ and tissue function. However, most samples are cultured and studied in unphysiological environments, without temporal variation in the biochemical cues that are ubiquitous *in vivo*. The artificiality of these conditions undermines the predictive value of cell culture studies. We ascribe the prevalence of this suboptimal culture methodology to the lack of practical continuous flow systems that are economical and robust. Here, we design and implement an expandable multiplexed flow system for cell culture superfusion. By expanding on the concept of the planar peristaltic pump, we fabricated a highly compact and multiplexed pump head with up to 48 active pump lines. The pump is incorporated into a custom, open-top superfusion system configured for conventional multi-well culture plates. We then demonstrated the utility of the system for *in vitro* circadian entrainment using a daily cortisol pulse, generating a sustained circadian amplitude that is essential for physiological emulation and chrono-pharmacological studies. The multiplexed pump is complemented by a package of fluidic interconnection and management methods enabling user-friendly and scalable operation. Collectively, the suite of technologies provides a much-needed improvement in physiological emulation to support the predictive value of *in vitro* biomedical and biological research.

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Development and utility of a microphysiological lung model for screening toxicity

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Current 3D *in vitro* models of the lung, which have primarily been developed using a transwell membrane scaffold with epithelial cells cultured at an air-liquid interphase (ALI), lack certain elements of the *in vivo* physiology, such as fluidic shear and endothelial cell components. We have developed a lung-on-a-chip model that mimics the small airway architecture and physiology. The device is comprised of a microfluidic chip consisting of a polydimethylsiloxane (PDMS) membrane, commercially available normal human bronchial epithelial cells, and maintains ALI across the epithelial cells. Additionally, the differentiated epithelium is surrounded by human primary microvascular endothelial cells. During the development phase, culture conditions including the timing of ALI growth of epithelial cells with endothelial cultures, co-culture media, and cell seeding density were optimized. The epithelial phenotypic assessments show the presence of mucus production, goblet cells, ciliated cells, and the formation of tight junctions. Several assays were developed to evaluate cytotoxicity endpoints including cell viability, oxidative stress, cell death, and biochemical measures such as ICAM-1, following exposure to TNF-α + IFN-γ (positive control) as part of the model development. Total particulate matter (TPM) from cigarette smoke was exposed apically at different concentrations for 4 hours and cell viability, reactive oxygen species (ROS), and cell death, were assessed 20 hours post-exposure, and epithelial permeability was assessed 1-hour post-exposure. Our results demonstrate a significant decrease in cell viability; and an increase in ROS, ICAM-1 expression, cell death, and epithelial permeability were observed. This small airway lung-on-a-chip offers a biochemically and physiologically relevant *in vitro* model to assess local lung perturbations caused by inhaled toxicants such as those present in cigarette smoke.

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Characterization of a rat and dog quad-culture liver-microphysiological system model

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Complex *in vitro* models (e.g., micro-physiological systems (MPS) or organ-on-a-chip models) often include physiologically relevant conditions like flow, shear stress, as well as multiple cell type interactions in 3D. Increasingly, there is an interest in evaluating these models for potential applications in preclinical drug development. Potential applications include use as an alternative model to *in vivo* studies for prediction of safety (or efficacy) of new investigational drugs. Unfortunately, vendors have largely ignored the production of animal cell based MPS models, focusing mainly on human models. However, MPS with cells from sensitive preclinical species could reproduce species-specific toxicity *in vivo* findings, which would provide significant confidence in the validity of human cell based MPS results and insight into the translatability of animal toxicity findings to humans. Keeping this in mind, we sought to establish and validate a protocol for building rat and dog quad-culture liver MPS models using the commercially available Emulate® platform (chips). The Liver MPS model included primary hepatocytes, sinusoidal endothelial cells, Kupffer cells and stellate cells and was characterized for multiple endpoints over a 14-day period. As a first step, we identified reliable sources for all four primary cryopreserved liver cell types for rat and dog and then successfully established a protocol for co-culture of these cells in physiologically relevant flow conditions. Model characterization included an evaluation of cell viability (LDH, ALT/AST release) as well as structural (bright field imaging and immunofluorescence), functional (albumin production) and metabolic (CYP and FMO activity) phenotypes. Initial study results demonstrated that our protocol and culture conditions enable building and maintaining high-quality rat and dog chips for up to 7 days. Starting after 7 days post-connection to flow conditions, we observed a gradual decrease in the viability of cells as well as in functional activity. Within our functions, this was the first effort to build an MPS model for preclinical species. The established liver MPS model is currently being evaluated as a more physiologically relevant alternative when compared to traditional 2D and simple 3D (spheroid) models in predicting species specific metabolite driven liver toxicity.

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Time- and spatially resolved readouts for organ-on-a-chip systems via non-invasive imaging techniques

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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. Conventional readouts, e.g., gene or protein expression assays, often lack in their capacity to fully capture these dynamic conditions as they often require destructive sample processing, do not allow for spatial resolution or only represent endpoint readouts. 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. Whereas FLIM is especially sensitive to metabolic changes by targeting the endogenous fluorophores NADH and FAD, RMS can access various cell and tissue structures due to their unique molecular-sensitive spectral fingerprints.

We implemented RMS and FLIM for OoC setups, in particular in a pancreas-on-a-chip and a beige-adipose-tissue model. 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. Moreover, FLIM enabled to investigate the metabolic balance between glycolysis and oxidative phosphorylation in tissue spheroids and was sensitive to detect early signs of apoptosis. In the Pancreas-on-a-Chip model, both techniques were able to monitor hypoxia- as well as glucose-induced changes on cellular metabolism and insulin secretion and resulted in equally sensitive detection yields, when compared to off-line bioassays.

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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Precise and fast control of the dissolved oxygen level in a lung tumor-on-chip

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In vivo tumor cells experience low oxygen levels (15 mmHg for lung cancer), called "hypoxia", as compared to "physioxia" in healthy tissue (40 mmHg for lung) (McKeown et al., 2014). This hypoxic environment is mainly due to the fast proliferation rate of tumor cells along with the creation of abnormal vasculature. Hypoxia promotes malignant progression and reduces drug efficiency. 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 (Nguyen et al., 2018). In parallel to the control of the extracellular matrix or cellular subtype composition, the on-chip oxygen control is also crucial to properly recapitulate *in vivo* tumor mechanisms. However hypoxic incubator is the most adopted technology, it comes with 2 major drawbacks: the lack of measurement of the oxygen level in the medium and the long equilibration time (more than two hours in conventional conditions). So far there are no commercial systems capable to reproduce gradients of oxygen inside microfluidic systems, mimicking not only global hypoxia, but also the fluctuations of local oxygen concentration due to angiogenesis and vessel leakages.

We present OXALIS (Oxygen ALImentation System), a new system to control the dissolved oxygen level in a microfluidic chip with unprecedented performance in terms of response time (200 sec), oxygen partial pressure accuracy (2 mmHg) and liquid flow control accuracy (0.1 µL/min). Oxygen cycles of a few minutes between physioxia and hypoxia can be reproduced. Moreover, this original approach also allows to measure *in situ* and continuously the cellular oxygen consumption, for various initial oxygen concentrations.

To reach a precise control in the chip, we have developed tumor-on-hip models with non-permeable materials (such as glass) for 2D and 3D cell culture using sliding wall technology. Thanks to this unique technology, we are currently studying the impact of slight pO2 variations on the expression of various HIF-1 target genes as well as on the cytotoxicity of paclitaxel in a lung tumor-on-chip model.

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Bioengineering services at Institut Pasteur

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The Biomaterials and Microfluidics core facility offers a wide range of bioengineering services to the researchers' community of Institut Pasteur. Our mission is to help our users overcoming technological bottlenecks in relation with cell-tissue organization and/or cell-microenvironment interactions. Practically, we provide the latest Organ-on-Chip technologies capable of recapitulating tissue physiology *in vitro*. This technology is particularly efficient for resolving various infectious processes while ensuring easy live imaging. Other functional assays such as permeability, metabolic activity and proteomic variations are also compatible with these chips. In parallel, we also have implemented a photolithography bench in order to allow for the design and production of custom microfluidic chips including bacteria trapping systems, Quake valves, cytokine gradient generators. The same tool could also be used for the efficient confinement of living cells/microorganisms either on micropatterns or by using topographically structured surfaces (microwells). On the Biomaterials, side we offer a wide range of synthetic 3D microenvironments in order to help with optimizing the production of organoids, spheroids, cysts and other polarized cell aggregates. This tool is very handy when investigating the impact of biophysical cues on cell organization. Finally, we are also investing in the development of a home-made laser photoablation station in order to create synthetic vascular structures in large 3D cell structures.

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Alternative method proposed for replacing the mouse anti-venom sera potency test

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Accidents from venomous animals are the first intoxication problem for humans in Brazil. In 2020, 30,891 cases were informed where 17,183 cases were related to Bothrops genus leading to 102 deaths and 2,253 Crotalus genus were informed, with 25 deaths. Sorotherapy is the only treatment available. The National Institute of Quality Control in Health, based in Rio de Janeiro, Brazil, uses near 5,500 mice/year for the potency assay of theses hyperimmune sera. This assay is based upon the death of animals. This study aims to develop an *in vitro* method for replacing this mice potency assay. The proposed method in based on venom soroneutralization exposed to a neuron cell line (SH-SY5Y, ECACC). Neuronal cells are exposed to raw Crotalus venom and to neutralized venom with antivenom (SAC) in order to evaluate the changing in curve. The first phase is being performed using a 2D neuron culture. In a second step, a 3D spheroids (neurons and astrocytes) will be used in a microfluidics system (organ-on-a-chip). The first results showed that neuronal cells markedly distinguish positive and negative control and that different venom concentrations show a linear response where higher concentration kills more cells than lower concentrations, when viability is tested by MTT. Next steps will be to evaluate the neutralized venom and work with spheroids in a chip with microfluidics system.

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Development of a microfluidic immunoassay for quality control of polyvalent pneumococcal vaccines

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Immunoassays, using membranes, are widely used for the identification of several chemicals, including several polysaccharide vaccines. However, this technique requires a long time of analysis and, in addition, they take several manual steps for execution. Microfluidic systems have been used to increase automation, reducing analysis time and amount of samples and reagents, which is paramount for vaccine quality control. In Brazil, the National Institute for Quality Control in Health (INCQS) is the institution responsible for batch-by-batch analysis of vaccines, complying with demands of the National Immunization Program (PNI). This work aims to develop a microfluidic system for the identification of polysaccharide vaccines present in polyvalent pneumococcal vaccines widely applied to the Brazilian population. The proposed methodology is the construction of a microfluidic system based on immunoassay membranes. The vaccine sample will be carried out into and evacuated from the microfluidic chip where the detection antibody will be conjugated to colloidal gold on the nitrocellulose membrane. Initially, polysaccharides 6B and 14, which are prevalent in Brazil, were tested using a conventional analysis system. The preliminary results were promising regarding the identification of vaccine polysaccharides. The next phase consists of in a construction of the microfluidic system detection. With this lab-on-a-chip system, it will be possible to simultaneously detect the ten vaccine polysaccharides present in the test vaccine. Furthermore, with this methodology, it will be possible to collect and identified polysaccharides for future quantification and characterization by physicochemical methodologies. This new system will reduce the analytical time, through the implementation of a micro-scale immunoassay, improving the binding kinetics of the antibodies, increasing the analytical sensitivity, besides reducing the amount of sample and reagent consumption in the analysis.

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A patient-derived iPSC liver acinus microphysiology system is an innovative precision medicine platform for optimizing clinical trial design of nonalcoholic fatty liver disease

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Non-alcoholic fatty liver (NAFLD) is the most prevalent chronic liver disease worldwide, characterized by fat accumulation in the liver ranging in severity from simple steatosis to steatohepatitis (NASH). NAFLD patients exhibit significant heterogeneity in phenotype and drug response favoring patient-specific therapeutic strategies. The *PNPLA3* rs738409 C > G (p.I148M), a widely reported SNP across genome-wide association studies, has a global frequency of 26.2%, categorizing it as the "common disease – common variant". Non-human liver disease models have inadequately reproduced human liver biology, making preclinical investigation in complex 3D *in vitro* models necessary to uncover disease mechanisms. Our goal is to develop an *in vitro* NALFD disease model using patient-specific iPSC-derived cells in the liver acinus microphysiology systems (LAMPS) that recapitulates key disease features enabling interrogation of targeted, patient-specific drug screening approaches.

We have recruited > 226 patients with NAFLD/NASH using the UPMC FLOW Clinic, with the goal of finding drug combinations that reverse the disease phenotypes associated with the variant population. Cohort genotype frequency analysis shows the variant presence significantly increases across disease severity. Functional studies in PNPLA3 I148M primary human hepatocytes show significantly higher intracytoplasmic lipid content. To best recapitulate the complexity of human liver physiology, the four-cell type LAMPS model was used to examine the impact of the variant on the progression of NASH disease. We investigate the differences of primary hepatocytes with iPSC-derived hepatocytes (iHeps) for both wild-type and variant carrying hepatocytes and their response to selective drug treatments on hepatic function and phenotype. Preliminarily, carrier primary hepatocytes accumulate more lipid content in the LAMPS model with disease-induced conditions which are consistent with published clinical data and *in vitro* studies. We are investigating more primary hepatocyte lots for both genotypes to validate our results. In parallel, we have developed and optimized a protocol for using iHeps in the LAMPS model and demonstrated recapitulation of liver physiology and hepatic maturity over eight days. The development of gene-edited variant iPSCs will complete our framework for the isogenic study of the variant on NASH progression allowing further investigation effects of patient-specific therapies.

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Microfluidic model recapitulates breast cancer metastasis using large tumor spheroids

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In breast cancer, patient survival rates drop from 90% to 27% once cancer cells metastasize to distant tissues. The major steps of the metastatic cascade include invasion, intravasation, circulation, extravasation, and colonization. Several factors dictate cancer cell dissemination, such as cytokine signaling and extracellular matrix (ECM) remodeling driven by stromal cells present in the tumor microenvironment (TME). Identifying the subpopulations of breast cancer cells that metastasize and understanding the biological and physical cues that regulate cancer cell dissemination is critical for therapeutic development, as 90% of cancer-related deaths are caused by secondary, not primary, tumors. To date, most *in vitro* breast cancer models use reductionist approaches to study a subset of the metastatic cascade and rely on single cancer cells suspended in ECM to model tumors, which lack physiologic relevance. Despite capturing biological complexity, existing *in vivo* models of breast cancer are hindered by cost, labor, and an inability to isolate and study specific factors that contribute to metastasis.

To overcome limitations of existing models, we engineered a comprehensive microfluidic model that allows visual monitoring of cancer cells sequentially navigating each step of metastasis. Our platform is engineered to incorporate large tumor samples, such as multicellular tumor spheroids or organoids, in ECM to model a primary TME with greater physiologic relevance. This primary tumor chamber is connected to downstream tissue sites through endothelialized fluidic channels that enable a route for cancer cell dissemination and metastasis. Currently, we are using our platform to in-

vestigate the role of tumor-associated endothelial cells and macrophages in promoting cancer cell dissemination and metastasis. Our microfluidic device is designed to allow anticancer therapeutics to be administered through blood vessel-like channels to target large, heterogeneous tumors growing in 3D ECM, which provides a more physiologically relevant approach to assessing the efficacy of small molecule and immune cell targeting therapies on inhibition of metastatic spreading. Future studies using this platform include testing the efficacy of NK cell and T cell therapies administered through blood vessel-like channels to target tumor growth and metastasis.

Presentation: Poster

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Development of 3D blood brain barrier-on-chip models: Applications towards increasing predictability of antibody-triggered receptor mediated transcytosis and neurotoxicity of CAR-T based immunotherapies

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The blood-brain barrier (BBB) is the most important biological barrier between the blood circulation and the central nervous system (CNS), it functions as a physical barrier and plays a major role as a transport and metabolic barrier. *In vitro* models of the human BBB are highly desirable for drug development and studies of neurovascular pathology. Human induced pluripotent stem cell (iPSC) derived brain endothelial-like cells (iBECs) have demonstrated a substantial advantage over primary and immortalized brain endothelial cells for BBB modeling.

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. We established iBEC microvessel lumens under physiological *in vivo* shear stress conditions (5 dynes/cm^2) in the endothelial channel of the chips, while human primary astrocytes and pericytes were cultured in the adjacent tissue channel separated by microfabricated 3 μm pores. We deployed this BBB-on-Chip model to study antibody-triggered receptor mediated transcytosis by perfusing the iBEC lumens with a well characterized single domain BBB-carrier FC5-Fc and non-crossing A20.1 control. Leveraging Wes (ProteinSimple), we established protocols for on-chip BBB permeability quantification, using anti-Fc and anti-His antibodies, in small sample volumes extracted from the microfluidic channels. We observed similar FC5-Fc transcytosis under 3D static conditions compared to conventional 2D transwell assays; however, 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 the SynBBB model 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.

Presentation: Poster

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Engineering vascularized mini-brain-chips for accelerating drug development

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Up to 1 in 6 people worldwide, 1 billion people, are estimated to be afflicted with a neurological disorder, costing over $500B in the US alone. For many neurological diseases there is still no pharmacologic treatment available that can slow or stop neuronal damage and the underlying molecular mechanisms for many diseases, such as Alzheimer's Disease (AD), remain unknown. A major bottleneck to developing effective therapeutics and understanding disease mechanisms is that current *in vitro* models fail to recapitulate human brain tissue with sufficient fidelity. The brain is comprised of 7 different cell types arranged in a stereotyped

3D architecture. Microphysiological systems harnessing natural biomaterials have enabled the co-culture of 3 of the relevant cell types into blood-brain barrier (BBB) models. However, many of these natural biomaterials have deleterious effects on neurons and have failed to support the co-culture of neurons with a BBB, let alone all 7 brain cell types. Engineered biomaterials provide highly tunable 3D scaffolds that can promote the formation of tissue mimics with physiologically relevant stiffness and biochemical compositions. Using a Cell-Instructive Material design strategy, we have engineered a soft hydrogel biomaterial with brain-specific biochemical cues that supports the self-assembly of all 7 brain cell types into a 3D mini-brain-tissue (miBrain). This miBrain 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 via single-cell RNA sequencing, mature blood brain-barrier markers, and functional interactions between cell types in real-time monitoring. This is the first vascularized mini-brain tissue model to our knowledge that contains all 7 brain cell types and with unprecedented level of biomimicry. We have further harnessed our model to probe the effect of APOE4, the strongest known genetic risk factor for sporadic AD. This miBrain model could be harnessed to probe disease mechanisms, assess pathological progression across patient-specific cohorts, and enable high throughput therapeutic screening for AD and other neurological diseases, all in a fast-forming, long-term enabled *in vitro* system that could hold potential for expediting drug discovery and development.

Presentation: Poster

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Engineering the hydrogel extracellular matrix composition for the salivary gland microbubble tissue chip

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Chronic dry mouth, or xerostomia occurs in > 50% of patients receiving radiation therapy for head and neck cancers due to off-target salivary gland damage, leading to loss of secretory function, and numerous chronic complications. Radioprotective drug development is imperative for preventing this highly debilitating condition. Salivary gland cells (SGCs) rapidly lose secretory acinar phenotype *in vitro*, likely due to disruption of the extracellular matrix during dissociation, which eliminates cues required for proper cell function.

To address this deficiency, SGCs were encapsulated and cultured in engineered extracellular matrices based on poly(ethylene glycol) (PEG) hydrogels. We combined this approach with microbubble (MB) array technology to develop a high throughput drug screening platform wherein cells are seeded in high density arrays of 15 nL spherical cavities molded in polydimethylsiloxane (PDMS). This system provides tunable control of the matrix microenvironment by alteration of cell-responsive, enzymatically-degradable peptide crosslinkers and presentation of small-peptide matrix epitopes. These studies focus on identification of hydrogel matrix conditions (degradation rate, matrix cue incorporation) that improve acinar cell phenotype and function and the functionality of the SGm platform.

To characterize gel degradation specific to SGm, we developed a method of fluorescent gel-labeling. Gels containing the "Fast" estimated degrading linker are completely degraded within 24 h, while gels containing the "Medium" linker are present to 48 h of culture. Within the SGm, our results indicate that longer duration of support provided by slower-degrading crosslinkers promotes sphere formation and structural organization, and may prevent overexpansion of non-acinar cells, thereby enriching the acinar cell population. Further, slow – degrading gels enhance the secretory response of SGm through 14 days of culture. Gels containing either the RGD, or IKVAV matrix epitopes generated smaller spheres than gels without epitopes, however, these appear to be connected. Longer culture durations favored epitope-containing gels, as these conditions yielded tissue mimetics with greater responsiveness to carbachol, with increased max fluorescence intensity, duration, % responsive tissue clusters, and reduced response latency. These results indicate that slower – degrading hydrogel matrices with matrix-mimetic epitopes appear to be critical to long-term SGm culture and promotion of the acinar phenotype.

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Transluminal endothelial bridging in a microfluidic model of primordial vessels

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Background: Angiogenesis is vital for tissue regeneration and repair. Best studied is sprouting angiogenesis (SA). However, intussusceptive angiogenesis (IA), wherein vessels regenerate by splitting, is critical for embryologic organ development and tumors. Furthermore, we recently discovered that IA is the major mode of angiogenesis during early-stage vascular regeneration in ischemic skeletal muscle (Arpino et al., 2021). This splitting process proceeded in primordial vessels with low shear stress. Moreover, the process was paradoxically accelerated by inhibition of VEGFR2, in contrast to SA. IA begins with the formation of a transluminal endothelial bridge, or pillar. The molecular and cellular mechanisms of this peculiar intraluminal event remain elusive because *in vitro* models have not been established.

Objective: To develop a live-cell model of endothelial pillar formation using low-flow microfluidics, and test the role of a VEGFR2-nitric oxide (NO) axis.

Methods: Microfluidic channels with a 100 µm by 100 µm cross-section were seeded with human umbilical vein endothelial cells (HUVECs) then perfused at low wall shear stress with or without chemical interventions. After 48 hours, cells were fixed and imaged with confocal microscopy at 300 nm resolution to generate 3D projections. Pillar content was quantified as a percentage of total cells.

Results: HUVECs circumferentially lined the channel and remained viable despite the ultra-low flow environment. Remarkably, transluminal bridges comprised of endothelial cell processes, or endothelial cell bodies including nuclei, were found at corners. Less than 2% of cells formed transluminal bridges. Inhibition of VEGFR2 with ZM323881 resulted in a 3.2-fold increase in bridging cells. Inhibition of NO synthase with L-NAME yielded a 3.1-fold increase in transluminal bridges. Supplementation of NO had no effect on baseline bridge formation but diminished the effect of the VEGFR2 inhibitor when infused concurrently.

Conclusions: We have developed the first live-cell model of transluminal endothelial bridging, the launching point for IA, and identified a novel role for a VEGFR2-NO axis in the process. These findings provide an important platform for studying and modulating this critical but poorly understood form of angiogenesis.

Reference

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Presentation: Poster

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Enhancing stereolithographic 3D-printing for the fabrication of bioanalytical microfluidics

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Digital light processing (DLP) 3D printing is a promising rapid fabrication method used for making clinical tests, lab-on-a-chip, and many other biomicrofluidics. These devices often require high levels of fabrication consistency and design complexity that can be difficult to achieve with many manual fabrication methods (i.e., soft lithography or glass etching), whereas 3D printing automation would simplify production.

The efficiency of 3D printing led many to believe the fabrication method would accelerate the use of microfluidics, but several obstacles slowed the ability of bioanalytical labs to fully utilize this technology. For commercially available printing materials, producing devices with microfeature print resolution is challenging. Additionally, cytotoxic components within many printing resins impact the ability to analyze biological cultures and low optical compatibility decrease options for on-chip imaging. Potential solutions to these problems are scattered throughout the literature and are rarely available in head-to-head comparisons, especially for use with biological samples and cells that are more sensitive to changes to the culture environment.

Therefore, we developed a concise guide to the principles of resin 3D printing with commercially available materials most relevant for fabrication of bioanalytical microfluidic devices. Intended to quickly orient labs that are new to 3D printing, the data-supported tutorial includes the results of selected systematic tests to inform resin selection, strategies for design optimization, and improvement of cytocompatibility of resin 3D printed biomicrofluidics.

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Presentation: Poster

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Spinal cord-chip from sporadic ALS patients reveals disease-specific biomarkers

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Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disorder, with the vast majority of cases being sporadic in nature with unknown genetic cause. ALS is characterized by progressive loss of cortical and spinal cord motor neurons, resulting in muscle weakness and death from respiratory failure typically within 3-5 years of diagnosis. Using induced pluripotent stem cell (iPSC) technology, patient-specific cells can be differentiated into cell types relevant to ALS. Microphysiological systems (MPS), also known as organ-chips, have microengineered three dimensional compartments that enable the co-culture of different iPSC-derived cell types to recapitulate conditions of human physiology *in vitro*. Here we developed an MPS-based model incorporating patient-derived spinal cord motor neurons (SC-Chip) to study young onset sporadic ALS. The SC-Chip top channel contains disease-relevant neuronal cells, and the bottom channel contains brain microvascular endothelial cells (BMECs). The porous membrane between the two channels allows for the establishment of a blood-brain barrier. The primary goal of this project is to establish robust and reproducible biomarkers of this disease by identifying metabolomic, transcriptomic, and proteomic signatures, as well as to test efficacy of candidate therapeutics to ameliorate disease-specific phenotypes. We have detected several biomarkers of sporadic ALS that are consistent across multiple studies in RNA-seq, proteomics, immunohistochemical staining and western blot analysis. These biomarkers were initially detected in a cohort of 5 patient-derived iPSC lines, compared to 5 control lines, and are currently being confirmed in an additional 10 ALS/ control lines. Identified biomarkers for sporadic ALS include increased expression of heavy, medium, and light chain neurofilaments as well as peripherin, an increase in calmodulin binding substrate neurogranin, and a decrease in neural RNA-binding protein ELAV3. We will next administer experimental therapeutics to the vascular side of the SC-Chip to determine (i) transport across the bloods-brain barrier and (ii) reversal of disease-specific phenotypes on the neuronal side.

Presentation: Poster

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Development of an advanced 3D printing system and an automated detection system for organs-on-a-chip research

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Organs-on-a-chip (OOC) system, or microphysiological system (MPS), is a new biomedical research field that aims to recapitulate organ-level tissue structures and organ functions for drug evaluation and disease modeling. In previous study, we have developed multiple OOC and MPS systems including blood vessels, heart, liver, tumor, etc. Our previous work demonstrated that the miniature organs made with advanced microfabrication, 3D printing, microfluidics, and tissue engineering techniques could form tissue-specific structures and could maintain desirable organ functions for more than four weeks.

In this work, we report our research and development of an ultra-high resolution two-photon 3D printing systems with ~150 nm resolution and millimeter scale fabrication ability for nanofabrication of OOC. Our customized novel setup allowed this system to print in parallel and construct of chip-body and internal fine patterns with significantly reduced fabrication time. We will also report construction of an automated "SMART" system for OOC imaging and analysis. This advanced 3D imaging system allows us to characterize OOC tissue and analyze its morphology and other functional features automatically and quantitatively. For example, this system could offer packaged solutions to analyze the tumor spheroids/organoid viability and invasiveness, together with the prediction of the drug classification and mechanism with the deep-learning based AI-algorithm, thus this system could be very useful for oncology drug screening and evaluation.

Presentation: Poster

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Construct a lung-on-a-chip system for disease modeling and toxicological studies

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Organ-on-a-chip, also known as microphysiological system, is to fabricate miniature organs on a chip at the size of a USB drive. Organ-on-a-chip technology involves creation of the microenvironment similar to corresponding human organs and co-culture of a variety of cells and tissues with defined structures, so as to simulate key functions of human organs *in vitro*. As a result, Organ-on-a-chip can be applied in drug screening, biological and medical researches, environmental safety evaluation, and disease modeling.

In the pandemic situation of covid-19, in order to build an instrument system for detection of virus transmission and virus infection, our research group used tissue-engineering technology and means to make a lung-on-a-chip model composed of human lung cells, and constructed a system that can control the liquid transmission among the chips. This model not only has the unique structure mimicking human alveoli and terminal bronchi, but also has some of the basic lung biological functions. We detected a series of important lung functional parameters, such as the permeability, the mucus secretion from alveolar epithelium, the change in permeability of pulmonary endothelial cells, and the inflammatory reactions in the lung-on-a-chip, that are similar to the physical and biological characteristics of human lung. The perfusion and transmission system can provide real-time observation and quantitative measurement of the spread of virus particles via aerosol, and the immune responses in macrophages, monocytes and endothelial cells in the system. Finally, we showed that such system can demonstrate the protection effect of surgical face mask and other protective equipment. In sum, this proposed platform should find broad applications in the future biological researches, and pharmaceutical studies, including evaluation of infectiousness Covid-19 and other virus/bacteria, and testing of efficacy for drug, antibodies, and other protective measures.

Presentation: Poster

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Organ on a chip database (OCDB): A comprehensive, systematic and characteristic organs-on-a-chip database

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Organs-on-a-Chip is a microfluidic microphysiological system that uses microfluidic technology to make high-resolution and real-time imaging analysis on the structure and function of living human cells at the level of tissue and organ *in vitro*. Compared with the traditional two-dimensional cell culture model and animal model, organs-on-a-chip technology can simulate the pathological and toxicological interactions between different organs or tissues more closely and reflect the collaborative response of multiple organs to drugs. Although lots of organs-on-a-chip-related literatures have been published, none of current databases have achieved all these functions: searching, downloading and analyzing data and results from literature of organs-on-a-chip. To address this need, we established a database named organs on a chip database (OCDB), as a platform to integrate information related to organs-on-a-chip from various sources: literature, patents, more than dozen public databases, many open access data of organs-on-a-chip and organoids, as well as the data generated by our lab itself. OCDB is composed of dozens of sub databases and modules and each sub database contains a number of data related to organs-on-a-chip, aiming to provide a comprehensive, systematic and convenient search engine for researchers. In addition, it provides functions such as the mathematical modeling, three-dimensional model and citation map to meet the needs of researchers and to promote the development of organs-on-a-chip. The organs on a chip database can be found in http://www.organchip.cn/

Presentation: Poster

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Biofabricated lung tissue models as assay platforms for drug development

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The COVID-19 pandemic has highlighted the need for validated, ready-to-use, and robust physiologically relevant 3D organotypic cellular assays to rapidly model the infectivity of emerging respiratory viruses and discover and develop new antiviral treatments. We have implemented a platform of validated human alveolar and tracheobronchial epithelial air liquid interface (ALI) tissue equivalents of increased physiological complexity and assessed their usefulness as *in vitro* assays in the context of live respiratory virus infections. We have established the cellular complexity of these lung epithelial ALI models, described SARS-CoV-2 and influenza virus infectivity rates and cell-type infectivity patterns, viral-induced cytokine production as it relates to tissue-specific disease, and demonstrated their pharmacologically validity as antiviral drug screening assay platforms. This platform of lung epithelial ALI tissue models include assays on transwell-based plate format and on high throughput, 64-chip microfluidic plates, which enable the inclusion of perfused vascularization to the lung respiratory track ALI tissues to further study the effects of viral infection on vasculature integrity. While these lung epithelial ALI tissue models are being used in the context of respiratory viruses' infection, their applicability expands into other diseases of the lung, including cancer, fibrosis, asthma, and COPD. Furthermore, the bioengineering methods used to create these tissues equivalents are modular and enable the assembly of complex tissues with tailored biological components and physiological properties, such as immune cells, which will further increase the disease relevance of these tissue equivalents for disease modeling.

Presentation: Oral

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Multiplexed microfluidic platform for stem-cell derived pancreatic islet beta cells

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The destruction or dysfunction of pancreatic islet β cells, source of the hormone insulin, lead to type 1 or type 2 diabetes, respectively. Exogenous insulin injection and islet transplant remain the gold-standard approaches to alleviate patients with diabetes. With the limited supply of cadaveric human islets versus the demand, stem cell-derived β cells offer an alternative for scientific discoveries as well as a potential surrogate for islet transplantation. However, the process of obtaining and maintaining functional stem-cell derived β cells is expensive and challenging. Coupled with the scarcity of primary islets, there is a need to develop better *in-vitro* culture systems for high-throughput discoveries of biomaterials, drugs, and cryopreservative agents for translational impact. Microphysiological systems (MPS) are promising *in-vitro* discovery platforms but scaling design schematics for high throughput screening and discoveries remain a challenge.

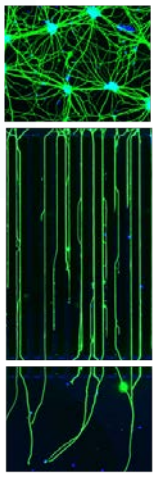
Here we present scalable schematic for a multiplexed islet MPS device that incorporates microfluidic gradient generators for parallelizing the culture conditions for compound testing with minimal number of beta clusters (5 vs gold standard of 50-100) per condition. The islet MPS recapitulates native physiological niche in which islet clusters are protected from shear forces by an endothelial-like barrier, while still providing a trickling flow to support long-term culture. We demonstrate the viability and functionality of the stem-cell derived beta cluster for a week, as assessed by the ~2-fold insulin release by the clusters to glucose challenge. To show the scalable multiplexing for drug testing, we demonstrate the exhaustion of islet clusters' insulin reserve after long term exposure to logarithmic concentration range of glybenclamide. The MPS cultured islet beta clusters also revealed a glycolytic bottleneck as inferred by insulin secretion responses to metabolites methyl succinate and glyceric acid. Thus, a scalable islet MPS platform is reported that can be used for biomedical and stem-cell discoveries.

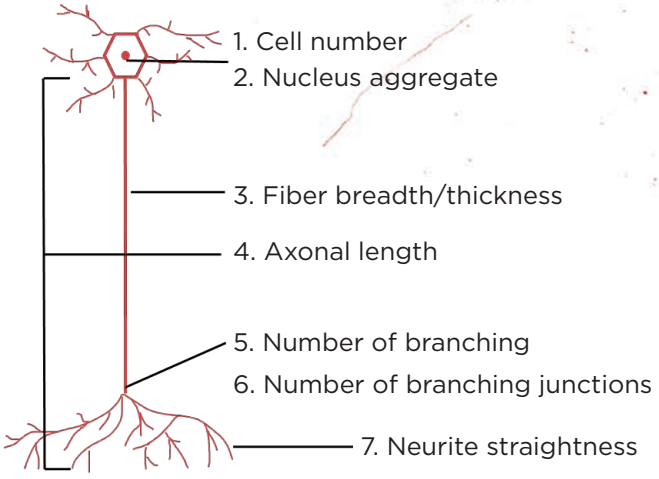
Presentation: Poster

NeuroHTS™ Data Profiler

Nervous system-on-a-chip: The next-generation neuronal assessment tool

NeuroHTS™: Data Profiler



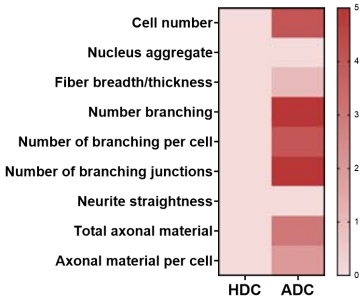


Big data approach to identify multiple neuronal markers in ONE assay

Compatible with:

- Biochemical profiling
- Genetic profiling
- Imaging

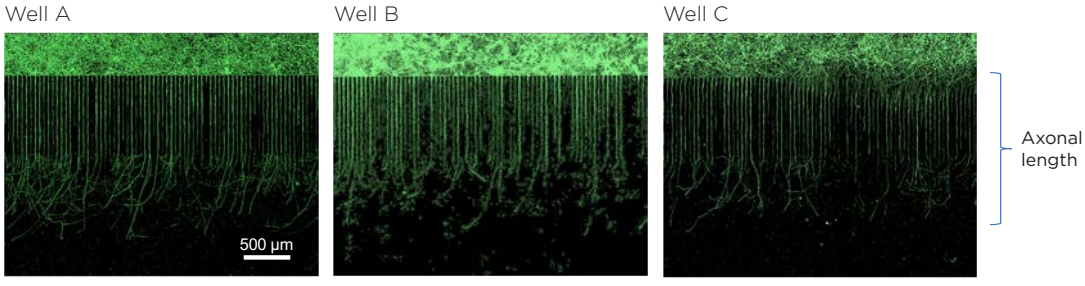
Sample heatmap comparing healthy cells and Alzheimer cells



Available models:

- Alzheimer's on-a-chip
- Parkinson's on-a-chip
- ALS on-a-chip

88% well to well reproducibility



Median axonal length = 1.7 mm | 1.6 mm | 1.6 mm

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