Human 3D Organ Models Networking Event
04. July 2024

AbstracTs
## Content

1. Identification of mechanisms of primary resistance to CHK1 inhibitors in BRAF<sup>V600E</sup> CRC PDOs
2. Stimulation of the synovitis using an animal-free synovial membrane model
3. Blood-brain barrier permeation in the autologous stem-cell derived four-organ-chip
5. In-Depth Characterization of Lung Organoids: An Imaging-Multi-Omics Approach
6. Highly biomimetic 3D bioprinted tubular small intestine model leads to in vivo-like differentiation of human adult stem cell-derived organoids
7. Towards a fully humanized IPSC-derived neural network for translatable cognitive drug screening: Developing a human 2D culture capable of synaptic plasticity
8. Manufacturing of Influenza-A specific ATMPs and their safety and efficacy testing in clinically relevant human models
9. The future of preclinical substance testing and disease modelling – The patient-on-chip
10. Decoding the role of histone modifier mutations in human neurodevelopmental disorder with multiOMICs analysis of hiPSC-derived 2D/3D co-culture models
11. Functional characterization of brain organoids using multi electrode array recording
12. Imaging of disease-related changes in the extracellular matrix in atherosclerosis: Characterization of three-dimensional in-vitro-models of inflammatory plaques
13. Functional and (ultra)morphological characterization of patient-derived organoids from head and neck cancer
14. Human Lung Organoids for Studying Host-Pathogen Interaction
15. Dexamethasone treatment of Parkinson’s disease derived brain organoids recapitulates phenotype of stressed transgenic mice
16. Evaluation of the Effects of Antimicrobial Hydrogels on Wound Healing Using a 3D Human ex vivo Skin Model
17. On real-time measurements of drug impact in a microphysiological system
18. Single nuclei RNA-seq profiling of primary human trophoblast organoids
19. Application of High Content Imaging to Investigate Cell Fate Decisions in Human Brain Organoids Subjected to OGD
20. In vitro models to study bacterial pneumonia
21. Contribution of human organ models to COVID-19 research. A large-scale systematic review
22. Utilizing CRC patient-derived 3D models to explore drug responses post KRAS inhibitor resistance
23. Brain organoid technologies to model human brain development
24. Unraveling causes of drug resistance in BRAFV600E CRC using single-cell mRNA sequencing of preclinical models
Developing a humanized astrocytic calcium imaging pipeline for compound screening

Characterization of resistance mechanisms and derivation of therapeutic approaches using organoids in cholangiocarcinoma

Elucidating SYNGAP1 Isoform Functions in Human Neurodevelopment Using Cerebral Organoids

Protocol for cancer cell purification during PD3D model development

Matrix-Free Human Organoid Monolayers Recapitulate Duodenal Barrier and Transport Properties

Developing humanized murine chronic brain slices as a 3D model of neurodegeneration

Rabep2 as an adaptable driver of vascular homeostasis

Generation of a human 3D bone model to mimic glucocorticoid-induced osteoporosis in vitro

The guardian of our airways: investigating molecular mechanisms of airway mucus clearance under inflammation

A model to study human intestine epithelium response to probiotics

Establishing a vascularized organ model on HUMIMIC chips

A breathable multi-compartment lung-on-chip model to study the (patho)physiological relevance of biological hydrogels in dynamic conditions

Toward a human stem cell-derived neuronal network for high-throughput cognitive drug screening: Characterizing functionality in hiPSC-derived neurons and brain organoids

A human Bone/Bone-marrow-on-a-chip system for preclinical investigation of new therapeutic approaches for autosomal recessive osteopetrosis

Towards Immunity on Chip – Immune Cell Perfusion of an IPSC-derived Intestinal Model

Human Gastrointestinal Organoids to Model Innate Immune Response and Infection

Light-inducible patterning of organoids: unraveling the role of WNT3A in human hippocampal development

Identifying Mechanisms of chemotherapy resistance in pancreatic cancer using organoid models

Magdeburg patient-individual, visceral organoid bank – a multi organ approach modeling tumor, tumor microenvironment and control tissue in primary and secondary malformations

A human iPSC-based platform for CRISPR-perturbations in liver systems

3D Bioprinted Perfusable Vascularized Organ Models via Sacrificial-Free Direct Ink Writing

Establishing a cholangioid library for biliary-niche-on-a-chip multicellular models for the study of ductular reaction

Defining inter- and intra-patient matrix heterogeneity of liver tissue and colorectal liver metastases

Primate-specific miRNAs in the context of neurodevelopment
Identification of mechanisms of primary resistance to CHK1 inhibitors in BRAF$^{V600E}$ CRC PDOs

Diogo Abreu$^{1,2}$, Stefania Klefaakes$^{1,2}$, Anna Kotarac$^{1,2,3}$, Lara Kunhardt von Schmidt$^{1,2}$, Alexander Malt$^4$, Alexandra Trinks$^5$, Markus Morkel$^{1,5,6}$, Natalie Bablitz$^6$, Ekaterian Korobkind$^6$, Ulrich Keilholz$^{1,3}$, Sebastian Stintzing$^{2,3}$, Naveed Ishaque$^4$, Christine Sers$^{4,6}$, Loredana Vecchione$^{1,2,3,7}$

1. Charité Comprehensive Cancer Center, Charité - Universitätsmedizin Berlin, Berlin, Germany
2. Department of Hematology, Oncology, and Cancer Immunology, Campus Charité Mitte, Charité - Universitätsmedizin Berlin, Berlin, Germany
3. German Cancer Consortium (DKTK), Partner Site Berlin, and German Cancer Research Center (DKFZ), Heidelberg, Germany.
4. BIH Center for Digital Health, Computational Oncology, Berlin Institute of Health (BIH), Berlin, Germany.
5. Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Biomedical Innovation Academy, Charitéplatz 1, 10117 Berlin, Germany
6. Institute of Pathology, Charité - Universitätsmedizin Berlin, Berlin, Germany
7. Berlin Institute of Health at Charité – Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, Charitéplatz 1, 10117 Berlin, Germany

BRAF$^{V600E}$ colorectal cancer (CRC) exhibit poor prognosis, especially in metastatic setting. Current standard systemic treatments, including target therapies, show a heterogeneous and poor response with poor overall survival, thus highlighting the need of new therapeutical options. Indeed, DNA damage inhibitors such as CHK1 inhibitors, might interfere with the necessity of cells to resolve the BRAF$^{V600E}$ oncogene-induced replication stress, thus leading to genome instability and synthetic lethality. From previous results, BRAF$^{V600E}$ CRC cell lines showed to be more sensitive towards CHK1 inhibition as compared to RAS mutated or wild type CRC cell lines. Similar results were obtained in BRAF$^{V600E}$ CRC PDOs, although more heterogeneous responses were observed. To understand this heterogeneous response and move towards a more personalized therapeutical approach in BRAF$^{V600E}$ CRC PDOs, we designed the current study. Here, we plan to perform a drug screen in BRAF$^{V600E}$ PDOs CHK1 resistant models in order to identify drugs that could synergize to these inhibitors. 10 models were tested for proliferation assay and western blot analysis upon CHK1 inhibition. We found 5 models to be sensitive to CHK1 inhibition, 3 to be intermediate sensitive and 2 to be resistant. Proliferation data were validated by western blot with apoptotic (cleaved-PARP), DNA damage ($\gamma$H2AX) and drug activity (paradoxical activation of CHK1 - S345 CHK1 phosphorylation) markers. Baseline scRNAseq, WES, RNAseq and Mass spectrometry data of those models is being analyzed, alongside scRNAseq data after drug perturbation in a resistant and sensitive model, to identify mechanisms of intrinsic resistance. The biomarkers identified through molecular analysis will be integrated with the data obtained from the drug screen to potential design molecularly driven trials in this setting of patients.
Stimulation of the synovitis using an animal-free synovial membrane model

Nour Akil, Moritz Pfeiffenberger, Frank Buttgereit, Timo Gaber, Alexandra Damerau

1. Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Berlin, Germany
2. Berliner Hochschule für Technik Berlin
3. German Rheumatism Research Center Berlin, a Leibniz Institute, Berlin, Germany

Rheumatoid arthritis (RA) and osteoarthritis (OA) are among the most prevalent forms of arthritis. If left untreated, both result in subchondral bone erosion, cartilage degradation, synovitis, and synovial fibrosis. Synovitis is characterized by synovial immune cell infiltration, lining layer hyperplasia, and activation of resident macrophages and fibroblast-like synoviocyte (FLS) subsets. This leads to releasing various pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α. Moreover, pathological mechanical stressors and the transforming growth factor (TGF)-β signaling pathway are linked to synovial fibrosis.

Here, we aimed to develop an in vitro animal-free, human-centered, 3D synovial membrane model that reflects the (patho-)physiology of the synovium to study the course of arthritis and test new therapeutic strategies.

Bone marrow-derived mesenchymal stromal cells (MSCs) were used to reflect the physiological state. Blood-derived monocytes were differentiated into alternatively activated macrophages (MLSs). MSCs and MLSs were incorporated into a synthetic RGD hydrogel and stacked layer-by-layer, mimicking the lining and sublining layer in an in vitro animal-free 3D model. Inflammatory processes were induced by TNF-α, whereas profibrotic processes were induced by stimulation with TGF-β for 72h. Therapeutic intervention with the TNF-α blocker adalimumab or 4-octylitaconate, which probably interferes with the TGF-β signaling pathway, was carried out from 48h. TNF-α stimulation increased the expression of pro-inflammatory genes such as IL6, IL8, and MMP1, which was attenuated by adalimumab. Furthermore, TGF-β stimulation increased α-smooth muscle actin expression at the protein level and the expression of TGFB at the gene level. However, 4-octylitaconate showed no significant effects within this model.

The study suggests that an in vitro human 3D synovial membrane model can replicate cytokine-driven cell- and matrix-related modifications by those found during arthritis. A quantitative histological analysis should be performed to make more precise statements at the protein level.
Blood-brain barrier permeation in the autologous stem-cell derived four-organ-chip

Beren Atac Wagegg¹, Leopold Koenig¹, Anja Hellwig¹, Uwe Marx¹,², Eva-Maria Dehne¹

¹. TissUse GmbH, Berlin Germany
². Technische Universität Berlin, Berlin Germany

Over the last years microphysiological systems have been increasingly accepted by academia and industry as a valuable tool in drug development to test substances for their safety and efficacy. Current systems still face the problems of heterogeneous tissue sources, hindering the development of patient specific chip systems. To overcome these limitations we established the four-organ-chip (Chip4) combining miniaturized autologous human intestine, liver, brain and kidney equivalents to study absorption, distribution, metabolism and excretion (ADME) derived from one single human induced pluripotent stem cell (hiPSC) line.

Understanding the ability to pass the blood-brain barrier (BBB) is crucial for assessing safety and efficacy in the development of neurological-active compounds. Therefore, we have enhanced our neuronal model by introducing blood-brain barrier specific endothelial cells (BBB-ECs).

Here, we present the adaption of the new neurovascular model to the Chip4. Furthermore, we show results of carbamazepine, propranolol and atenolol and their metabolization and permeation across the model of the BBB. The neuronal models were produced in a large-scale fully defined and controlled DASbox® bioreactor system and then cultured in an insert model with state of the art hiPSC-derived BBB, to separate the neuronal compartment from the rest of the system. The BBB-ECs form a tight monolayer on the Transwell membranes as shown by trans-endothelial electrical resistance and low sodium fluorescein permeation and maintain their phenotype in the Chip4. Furthermore, metabolization and permeation characteristics of the compounds were comparable to the in vivo situation underlining the high physiological relevance of the system.

Acknowledgment

The presented work is supported by public funding from the German Ministry for Education and Research BMBF (HiPSTAR, funding code: 01EK1608) and European Union’s Horizon 2020 research and innovation program under grant agreement No 964537.
Co-culture of metabolic active liver model and patient-derived gastric cancer in an automated multi-organ-test platform using 5-FU prodrugs for enhanced standardization.

Ricky Bayer\textsuperscript{1,2}, Cristina Brischetto\textsuperscript{1}, Sina Bartfeld\textsuperscript{1}

1. Medical Biotechnology, Institute for Biotechnology, Technische Universität Berlin, Berlin, Germany
2. TissUse GmbH, Berlin, Germany

Gastric cancer, the third deadliest cancer globally, causes about 780,000 deaths annually. The treatment often involves 5-fluorouracil (5-FU), a fluoropyrimidine anticancer agent. However, 5-FU is associated with poor bioavailability, severe toxicity, and low patient tolerance. To address these issues, oral 5-FU prodrugs like Tegafur and Capecitabine have been developed for targeted therapy.

Hepatic biotransformation is essential for activating these prodrugs and targeting cancer cells effectively. Thus, a preclinical model enabling cross-organ communication is crucial for studying anticancer prodrugs in vitro. While patient-derived organoids (PDOs) replicate many tumor characteristics, they lack systemic liver connections. Multi-organ chips (MOCs) offer a platform to study drug metabolism by mimicking organ functions. Combining 3D tumor organoid culture with microfluidic chip technology can enhance organ function and sensitivity to stimulation.

This study aimed to show the benefits of automated multi-organ chips over standard 3D culture for evaluating drug efficacy and reproducibility of clinical responses. Gastric tumor-derived organoids were cultured with a liver model using human hepatoma cell line HepaRG and human hepatic stellate cells (HHSteC) in a microphysiological system (HUMIMIC Chip2, TissUse GmbH). The HUMIMIC AutoLab (TissUse GmbH) system automated the prodrug application. Viability, apoptosis, and metabolic activity were assessed using viability assays (CellTiter-Glo 3D, Promega), immunofluorescent, and metabolic analyses (glucose consumption, lactate release, and lactate dehydrogenase release). In addition, the experiments were performed in two separate laboratories to test the reproducibility through standardization to verify results.

Automated multi-organ chips could significantly enhance preclinical drug efficacy and metabolism testing in target organs, providing reliable data for clinical trials.
In-Depth Characterization of Lung Organoids: An Imaging-Multi-Omics Approach

Erik Becher¹, Julia Dreimann¹, Zeynep Demir¹, Artür Manukyan², Izabela Plumbom², Maren Mieth¹, Katharina Hellwig¹, Elena Remacha¹, Li-Ling Yang¹, Doris Frey¹, Sandra Kunder¹, Anne Voß¹, Mara Fischer¹, Anna Löwa¹, Morris Baumgardt¹, Achim D. Gruber³, Thomas Conrad², Altuna Akalin², Stefan Hippenstiel¹, Andreas C. Hocke¹

1. Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité - Universitätsmedizin Berlin, Berlin, Germany
2. Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine, Berlin, Germany
3. Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany

Understanding the characteristics of human lung organoids is essential for their use in mimicking the human lung and facilitating the investigation of its (patho-)physiology and host-pathogen interactions. However, their constant development and increasingly complex cellular and structural composition intensify existing challenges, such as donor and batch-to-batch variability. To address these challenges, it is necessary to quantify organoid complexity and heterogeneity at the molecular, cellular, and morphological levels in a standardized manner.

Here, we present an integrative approach to characterize adult stem cell (ASC)-derived human lung organoids from ten donors, over thirteen passages and nine months of culture, using imaging, image segmentation, single-cell, and spatial transcriptomics methods. We employ multiplex imaging, high-content scanning, and semi-automated image segmentation to analyze the cellular composition by characteristic cell markers established in reference lung samples. This reveals not only the cellular composition but also morphological characteristics such as organoid size, number of cells, and number of lumens per organoid. These 2D parameters from histological sections are compared to both 3D fixed and live organoid samples imaged using light sheet microscopy. Furthermore, we analyze molecular and cellular composition via RNA single-cell sequencing and spatial transcriptomics. For the latter, we present cell type annotation in lung and organoid samples, enabling spatial cell-specific analysis at the transcriptome level.

With the analysis pipelines presented here, organoid researchers across all organ fields can achieve comprehensive characterization of their organoids at the protein, transcriptome, and structural levels. This approach not only enhances our understanding of the organoid model in use but also paves the way for the development of more advanced organoids and their reliable and reproducible applications in research and medicine.
Highly biomimetic 3D bioprinted tubular small intestine model leads to in vivo-like differentiation of human adult stem cell-derived organoids

Alessandro Bentivogli, Konrad Schmidt1, Ahed Almalla4, Marie Weinhart4,5, Pilar Samperio6, Sina Bartfeld6,7, Sarah Hedtrich1,2,3

1. Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Center of Biological Design, 13125 Berlin, Germany
2. Department of Infectious Diseases and Respiratory Medicine at Charité – Universitätsmedizin Berlin, Germany
3. Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC V6T 1Z3 Canada
4. Institute of Chemistry and Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany
5. Institute of Physical Chemistry and Electrochemistry, Leibniz Universität Hannover, 30167 Hannover, Germany
6. Institute for Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany
7. Research Centre for Infectious Diseases, Institute for Molecular Infection Biology, Julius Maximilians Universität Würzburg, 97020 Würzburg, Germany

In recent years, in vitro models have gained popularity as a fast and effective way to better understand disease pathogenesis and to further drive progress in therapeutics. Specifically, intestinal models with high in vivo-fidelity could provide important support in tackling some of the most common disorders and shed light on key pathways linking together different parts of our body in healthy and diseased states. However, current solutions fail to aptly recapitulate the different traits found in the human gut, be that due to the absence of the various intestinal cell types, lack of geometrical, cellular or mechanical cues or missing peristalsis-like flow and air-liquid interface conditions.

Herein, we developed a human-derived 3D small intestine tissue model through microfluidic bioprinting of single-layered hollow tubes using decellularized porcine small intestinal submucosa and sodium alginate that is functionally and structurally comparable to its native counterpart. This coaxial bioprinting technology exploits the instantaneous ionic crosslinking between sodium alginate and calcium chloride resulting in strong and stable hollow tubes with an average inner diameter of 500 μm and average wall thicknesses of 60 μm. Our 3D bioprinting approach allows us to recapitulate not only the protein composition of the substrate found in vivo but also the tubular geometry of the human intestine. Moreover, this extrusion system allows us to obtain a continuous high-throughput fabrication of perfusable tubes that can be adjusted in length and cross-sectional area. We leveraged these highly biomimetic structures to induce the formation of an epithelial tube-shaped monolayer from adult stem cell-derived intestinal organoids, thus proving the key role that scaffold geometry plays in stem-cell differentiation. The potential of our approach opens the door for more complex tissue constructs that can include additional physiological and diseased stimuli such as flow-induced shear stress, cell-cell interactions and multi-organ-on-chip systems.

References
Towards a fully humanized IPSC-derived neural network for translatable cognitive drug screening: Developing a human 2D culture capable of synaptic plasticity

Larissa Breuer1,2, Katarzyna Ludwik1,3, Jeremy Krohn1,3, Harald Stachelscheid4,5, Camin Dean1,3

1. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Berlin, Germany
2. Freie Universität Berlin, Germany
3. Bernstein Center for Computational Neuroscience (BCCN) Berlin, Germany
4. Berlin Institute of Health (BIH) at Charité, Berlin, Germany
5. Charité - Universitätsmedizin Berlin, Berlin, Germany

With the prospect of a better translation compared to mouse-derived systems, hIPSC-derived neuronal networks are a promising model system for overcoming a failing system of cognitive drug development in the CNS disease field. This, however, requires a network capable of synapse strengthening and weakening, which is supported by human astrocytes and microglia: A fully human model of a minimal brain circuit. Human hIPSC-derived iNeurons cultured for a sufficiently long time express pre-synaptic markers and show activity in multi-electrode recordings in many reports. But convincing post-synaptic immunostainings are rare, as are functional assays investigating pre- and post-synaptic function and maturation.

In our hIPSC-derived NGN2-iNeurons co-cultured with mouse astrocytes based on Zhang et al. 2013 (1) we see PSD-95 and NR-1 colocalizing with presynaptic terminals at 45 days or 71 days in vitro, respectively, after plating them as NSCs. These iNeurons also show spontaneous and evoked calcium responses. In addition, we used FM1-43, a fluorescent styryl dye that inserts into the membrane and reports synaptic vesicle recycling, to assess synapse function. Our iNeurons show exponential dye release kinetics in response to stimulation, indicating mature synaptic vesicle release, but only in co-culture with astrocytes. To replace mouse astrocytes with human astrocytes, we created and characterized a stable doxycycline inducible SOX9-NFIB IPSC line, based on Canals et al. 2018 (2), which matures to functional astrocytes that can reliably support iNeuron cultures.

We aim to standardize a fully human IPSC-derived brain cell network with mature neurons that develop dendritic spines capable of synapse strengthening and weakening that is supported in co-culture by IPSC-derived astrocytes and microglia.
Manufacturing of Influenza-A specific ATMPs and their safety and efficacy testing in clinically relevant human models

Lisa-Marie Burkhardt1, Anna Löwa1, Lukas Ehlen1, Niklas Wiese1, Claudia Beltran-Mestres2, Ugarit Daher1, Melanie Rothe1, Anna-Catharina Krebs2, Janine Arndt1, Mathis Hertel1, Andy Römhild1, Stephan Schlickeiser9, Mir-Farzin Mashreghi1, Michael Schmück-Henneresse2, Hans-Dieter Volk1, Petra Reinke1, Andreas Hocke4, Leila Amini1,2


Immunocompromised patients such as patients after solid organ transplantation (SOT) are commonly treated with immunosuppressive drugs to circumvent organ rejection. This entails patients being equipped with a strongly diminished endogenous T cell response against pathogens e.g. viruses. Recurrent viral infections with seasonal Influenza A virus (IAV) and other viral pathogens represent major health concerns for transplant patients, since immunosuppression leads to unresponsiveness to vaccines, and viral complications can lead to organ rejection and high mortality, all of which cannot be adequately combated with state-of-the-art antiviral drugs.

To regenerate a long-term, protective T cell response in immunocompromised patients, we have focused on manufacturing autologous and partially-matched IAV ATMPs preparing for an off-the-shelf approach. Our next-generation manufacturing protocol includes a GMP compliant process, allowing scalability using GRex bioreactors. General functionality is evaluated by read-outs including effector cytokine production and target specific killing steered by a pool of conserved and seasonal viral peptides. We further implement in-depth sequencing, proteomics and off-target analysis, which is acquired to prove safety for clinical translation. Since animals are no natural hosts for respiratory viruses and critical evaluation of cellular therapeutics shows the well-known complications in mice, we need new models showcasing not only failure prediction but also efficacy parameters. In our cooperative research project, we gained first insights on IAV ATMPs and their safety and efficacy using a human lung model enabling native IAV infection. First data showed no detectable allogenicity even under partial matching conditions as well as cytotoxicity towards infected tissue. Our immunocompetent model will further pave the way for defining matching criteria for off-the-shelf anti-viral ATMPs. With this, we envision to take a step further, towards human based ATMP testing, which is a requisite for clinical reproducibility and more and more accepted by regulatory agencies.
The future of preclinical substance testing and disease modelling – The patient-on-chip

Eva-Maria Dehne1, Beren Atac1, Anja Patricia Hellwig1, Sophie Rigal1, Juliane Hübner1, Reyk Horland1, Uwe Marx1,2

1. TissUse GmbH, Oudenaarder Str. 16, 13347 Berlin, Germany;
2. Technische Universität Berlin, Department of Medical Biotechnology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

Microfluidic systems have shown to be a powerful tool for recreating tissue- and organ-like functions, providing the basis for developing preclinical assays with improved predictive power. Especially, platforms co-culturing several human micro-tissues in a physiologically relevant arrangement could provide a translational solution.

The HUMIMIC Multi-Organ-Chip platform is capable of maintaining and culturing miniaturized organs emulating the biological function of their respective full-size counterparts over long periods. Major biological features such as pulsatile fluid flow, efficient nutrition, and physiological tissue-to-fluid and tissue-to-tissue ratios can be incorporated. Moreover, the platform supports the development of a range of testing needs, including repeated dose applications up to at least 28 days. Several selected industry-tested assays ranging from single-organ to four-organ systems will be presented. Respective fields of application range from DMPK, safety and hazard identification to mode of action and efficacy studies. Furthermore, efforts to generate a patient-on-chip mimicking healthy or diseased phenotypes using patient specific engineered tissue are shown. This allows to generate individualized risk assessment data or develop personalized treatment options with the patient’s specific disease background.

The system’s robustness as well as its capacity to provide in vivo relevant information about exposure scenario-dependent changes in bioavailability will be evaluated. New application opportunities as well as important challenges in realizing the full potential of this technology will be addressed to advance the preclinical testing and disease modelling field.
Decoding the role of histone modifier mutations in human neurodevelopmental disorder with multiOMICs analysis of hiPSC-derived 2D/3D co-culture models

Charlotte Dresen1, Sarah Green1, Souhaila Wüsthoff1, Tatjana Luganskaja1, Thadoe Thukha1, Daria Bunina1

1. Max Delbrück Centre for Molecular Medicine (MDC)

The intricate development of the human brain requires precise epigenetic regulation of cell differentiation. Histone-modifying enzymes hold a key role in managing chromatin accessibility and transcription factor recruitment during brain development, as evidenced by the increasing number of mutations in chromatin modifiers linked to human neurodevelopmental disorders (NDDs) like ASD and Kabuki Syndrome. Remarkably, patients carrying mutations in modifiers of histone 3 lysin 4 (H3K4), including the histone demethylase LSD1 and methyltransferases MLL1/MLL2, often also present with congenital cardiovascular pathologies.

This unexpected correlation between neural and vascular defects suggests that H3K4-modifiers might regulate both cell fates in development. With H3K4-mono-methylation mainly found at transcription-activating enhancer sites, we hypothesize that the mutations cause changes in chromatin dynamics, disrupting gene regulatory networks relevant to both neural and vascular differentiation. While a recent MLL2-KO study in zebrafish points to severe vascular defects in brain development, it is unclear whether similar phenotypes exist in a human model system.

To address these questions, we utilize single-cell multiOMIC techniques on hiPSC-derived 2D/3D neurovascular mono- and co-culture models, including cortical brain organoids, iNeurons (NGN2) and iEndothelial Cells (ETV2), cardiomyocytes and vessels on a chip. With this approach, we aim to uncover shared gene-regulatory networks governing neural and vascular specification in brain/cardiac development and try to disentangle neural and vascular contributions to pathologies of H3K4-modifier associated NDDs.

Functional characterization of brain organoids using multi-electrode array recording

Hanna Dubrovska\textsuperscript{1,2,5}, Miriam Wandres\textsuperscript{3,4}, Agnieszka Rybak-Wolf\textsuperscript{3,4}, Camin Dean\textsuperscript{1,5}

1. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Berlin, Germany
2. Freie Universität Berlin, Germany
3. Max Delbruck Center (MDC) Berlin, Germany
4. Berlin Institute of Health (BIH) at Charité, Berlin, Germany
5. Charité - Universitätsmedizin Berlin, Berlin, Germany

Animal models have enabled numerous discoveries in basic science and drug development. However, there is a great translational gap between animal and human systems for development of effective drugs, especially in the case of brain disorders. Only few effective treatments developed in animal models show similar effects when tested in humans. This issue together with the 3R and ethical considerations has facilitated a shift from animal to humanized models in biomedical sciences. An ever-growing number of new human iPSC-derived cellular (2D) and organoid (3D) systems are being created and used not only in basic research, but also in drug development, and especially in neuroscience research. 3D brain organoids in particular, more closely resemble the complex structure of the human brain.

Previously, brain organoids have mainly been characterized structurally and on the transcriptomics level. Although these studies show promising results and similarity of organoids to the brain cell composition of the human brain, a functional readout of brain organoids, especially in terms of synaptic plasticity, is still lacking.

To fill this gap we are characterizing the electrophysiological properties of iPSC-derived brain organoids using multi-electrode arrays (MEAs). We find that brain organoids greater than 100 DIV show spontaneous activity that is increased by the voltage-gated potassium channel agonist 4-aminopyridine (4-AP), and blocked by TTX. We are now testing different types of brain organoids and their responses to 4-AP and chemical LTP (long-term potentiation). This will help further validate the use of brain organoids as a humanized model for basic research and drug development.
Imaging of disease-related changes in the extracellular matrix in atherosclerosis: Characterization of three-dimensional in-vitro-models of inflammatory plaques

Nike Fiebig1,2, Tim Setzkorn1,2, Lena Kampen1,2, Andrea Weller1,2, Anke Stach1,2, Amani Remmo3, Wolfram Poller4, Antje Ludwig1,2

1. Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany
2. Department of Cardiology, Angiology and Intensive Care Medicine, Deutsches Herzzentrum der Charité, Charitéplatz 1, 10117 Berlin, Germany
3. Physikalisch-Technische Bundesanstalt Braunschweig und Berlin, Abbestraße 12, 10587 Berlin, Germany
4. Harvard Medical School and Massachusetts General Hospital, 55 Fruit St, Boston, Massachusetts 02114, US

Developing non-invasive methods for early detection of unstable atherosclerotic plaques is crucial for preventing life-threatening complications like myocardial infarction and stroke1. One promising approach involves the use of magnetic nanoparticles (MNP), which have shown potential in targeting and visualizing atherosclerotic lesions2. Experimental evidence suggests interactions with glycosaminoglycans (GAGs) in the extracellular matrix (ECM) influence the uptake of MNP in atherosclerotic lesions. To reduce animal testing, we present a 3D multicellular microtissue model replicating atherosclerotic tissue and ECM modulation.

To investigate this, the spheroid model was selected as a suitable model as it allows particularly effective analysis of the ECM. Spheroids were grown from human umbilical artery smooth muscle cells (HUASMCs) and endothelial cells (HUAECs) and co-cultured with THP-derived foam cells. Cryo-sectioned spheroids underwent histochemical analysis for ECM composition, compared with aortic root sections from LDLR−/− mice on a high-fat diet to assess the atherosclerotic conditions of the model against in vivo plaques. Additionally, spheroids from HUASMCs and from both HUASMCs and HUAECs were incubated with the citrate-coated MNP Synomag® and analyzed using magnetic particle spectroscopy (MPS) and Prussian blue staining.

The dimensions of the spheroids matched those of fibroatheromas in the aortic root of mice, exhibiting a similar ECM composition with high GAG expression and low collagen expression. Prussian blue staining revealed a higher uptake in spheroids generated from both cell lines than from only HUASMCs which is also consistent with the measured MPS data.
Functional and (ultra)morphological characterization of patient-derived organoids from head and neck cancer

Anne-Sophie Fisch 1, 2, Johanna Plendl 2 and Ingeborg Tinhofer-Keilholz 1

1. Department of Radiooncology and Radiotherapy, Charité University Hospital, Berlin, Germany Berlin Institute of Health (BIH) at Charité, Berlin, Germany
2. Department of Veterinary Medicine, Institute of Veterinary Anatomy, Freie Universität, Berlin, Germany

Intrinsic and environmental tumor characteristics increase therapy resistance, resulting in treatment failures and high recurrence rates in human head and neck squamous cell carcinomas (HNSCC). This study aims at the (ultra)morphological and functional characterization of patient-derived organoids (PDOs). It explores their applicability to investigate individual resistance mechanisms to radiation.

Ultrastructural features of two PDOs and the changes upon irradiation were explored with transmission electron microscopy. Hypoxia was assessed using Pimonidazole in one PDO. ATP-dependent viability, clonogenic survival and organoid volumes were used as functional read outs for radiosensitivity in three models. Immunofluorescent gH2AX foci were stained for visualization of DNA double strand breaks in one PDO. Cell death and proliferation was assessed using live dead fixable green reagent and Ki67 with confocal fluorescence microscopy.

High nuclear to cytoplasmatic ratios, prominent cell organelles and wide intercellular spaces, correspond to the ultrastructure of poorly to moderately differentiated squamous cell carcinomas. Upon irradiation, cells in the outer rim of the organoid formed protective barriers and ejected cell debris in an organized manner. Interestingly, extent of damage between organoids was heterogeneous within the same model. Similar to this observation was the abundance of proliferative and dead cells. Compared to initial gH2AX foci, assessed 1 hour after irradiation, fewer foci were detected after 24 hours, due to functioning DNA repair mechanisms. The inner core of several organoids had a positive fluorescence signal for hypoxia. PDOs showed a dose-dependent decrease in metabolic activity, proliferation and clonogenic survival, and a considerable interpatient variability in survival.

PDOs might be valuable models to investigate individual responses to radiation therapy and the molecular mechanisms underlying radioresistance. In future experiments, we plan to establish a protocol for the automated quantification of gH2AX in organoids. Furthermore, we will investigate the impact of hypoxia on radiosensitivity of the models in more detail.
Human Lung Organoids for Studying Host-Pathogen Interaction

Mara Fischer, Anna Löwa, Morris Baumgardt, Katja Hönzke, Zeynep Demir, Erik Becher, Maren Mieth, Diana Fatykhova, Julia Dreimann, Josephine Melzer, Katharina Hellwig, Doris Frey, Stefan Hippenstiel, Andreas C. Hocke

Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité – Universitätsmedizin Berlin, Berlin, Germany

Lower respiratory tract infections, despite antimicrobial and adjunctive treatments, still cause high disease burden in patients. To understand the mechanisms by which human respiratory pathogens such as influenza A virus (IAV) and Streptococcus pneumoniae (S. pneumoniae) induce damage in the lung and to develop new countermeasures, innovative models are required. To this end, our lab extensively used ex vivo lung tissue and has established organoids derived from human adult stem cells (ASC). We aimed to apply a variety of organoid-derived models to study infections: To target the apical side of the cells for pathogen adhesion, organoids were either shredded or microinjected (apical-infection), or cultured in suspension to achieve an apical-out phenotype (apical-out infection). Additionally, we introduced organoid-derived monolayers for 2D infection. We used IAV, S. pneumoniae, and proinflammatory cytokines to measure infection characteristics and cellular responses, such as pathogen replication, cytokine response, and, via live cell imaging in a 2D setup, apoptosis induction. Furthermore, we successfully applied inhibitors to reduce viral replication. Suspension culture allowed a more productive viral infection than organoid shredding, which is mainly useful for pathogens with exclusively apical host cell entry. Data of our ex vivo lung tissue model enable the validation of the results obtained from organoid-derived infection models. In applying the above-mentioned methods, we are continually evolving a robust human lung model system for investigating complex (patho-) physiological mechanisms.
Dexamethasone treatment of Parkinson’s disease derived brain organoids recapitulates phenotype of stressed transgenic mice

Silke Frahm1, Vivian Schulz1, Carolin Genehr1, Maren Wendt1 Franz Theuring1, Sebastian Diecke1

1. Stem Cell Technology Platform, MDC, Berlin, Germany
2. Charité - Universitätsmedizin Berlin, Berlin, Germany

Human iPSC derived brain organoids are increasingly being used as 3D models of neurodegenerative disorders. However, this new technology still has limitations, such as the lack of immune and vascular cells. Therefore, it is essential to verify the manifestation of relevant pathological aspects in 3D models e.g. by comparing data obtained from transgenic animals with human organoids that share the disease causing mutation. Here, we examined the effect of chronic stress in three models overexpressing human α-synuclein (a-syn): a transgenic mouse model (h-a-synL62) and brain organoids as well as iNeurons derived from iPSCs of a Parkinson’s disease (PD) patient. We found that a-syn expression is differentially modulated by glucocorticoids and detected a pronounced glucocorticoid receptor (GR) deficiency in stressed mice. We validated our in vitro model and showed that the differential effect of stress on a-syn and GR levels can be reproduced in cerebral organoids generated from healthy and PD diseased donors after treatment with the synthetic GC Dexamethasone (Dex). Further, PD organoids and iNeuron cultures showed increased gene expression of inhibitory interneurons as well as an excitatory-inhibitory imbalance measured in a multi-electrode array, which was restored to control levels upon dex treatment. In summary, we established and validated a human 3D culture model that can be used to study particular molecular and functional alterations associated with neurodegenerative diseases, offering replacement of research animals.
Evaluation of the Effects of Antimicrobial Hydrogels on Wound Healing Using a 3D Human ex vivo Skin Model

Xiao Guo1, Ulrike Blume-Peytavi1, Annika Vogt1, Fiorenza Rancan1, *

1. Clinical Reasearch Center for Hair and Skin Science, Department of Dermatology and Allergy, Charité - Universitätsmedizin Berlin, Berlin, Germany

Our previous study has demonstrated that human ex vivo full-thickness skin serves as robust platform for dermatological research, providing a controlled environment to test skin-drug interactions, without using live animals. In this study, ex vivo skin was cultured for 12 days in six-well plates with trans-well inserts to simulate superficial wounds. Various antimicrobial peptide-based hydrogels were topically applied to evaluate their effect in wound healing, with untreated skin as the control. Results indicated only a moderate time-dependent increase in lactate dehydrogenase (LDH) activity, which reflected skin cell viability. Histological observations showed from day 6, OCT, Gel A and Gel B treated wounds exhibited delayed healing compared to the complete closure in untreated wound by day 12. These observations were consistent with in vitro results where medium collected from macrophages treated with hydrogels (notably OCT and Gel A) exhibited toxic effects and delayed scratch wound closure in fibroblasts. Furthermore, the vascularization and re-epithelialization processes were assessed with CD31 and keratin-17 (K17) immune-staining, respectively. While CD31 expression was noted in all samples, with the highest expression observed on day 12 in Gel A and Gel B, K17 expression was markedly absent, except for untreated wound by day 12. Analysis of skin extracts revealed a higher level of growth factors (HGF, FGF-basic, VEGF, TGF-β1) in Gel A and Gel B, potentially correlating with improved revascularization as indicated by enhanced CD31 staining. Meanwhile, moderate increases in immunomodulatory cytokines and MMP2 were detected in Gel A treated samples. As for analytes tested in medium, significant increases in IL-1α were observed in Gel A and Gel B treated groups on day 6, with Gel B also showing enhanced MMP-2 activity. These findings demonstrate human ex vivo skin act as an effective pre-clinical platform for exploring the effects of topical treatments on wound healing process.
On real-time measurements of drug impact in a microphysiological system

Christian Gürnth-Marschner, Marie Flechner, Jürgen Loskatov, Ulrike Pfohl, Christian RA Regenbrecht, Lena Wedeken, Katja Uhlig

Physiologically relevant in-vitro tumour models increase the success rate of new pharmaceuticals, reduce the number of animal testing and result finally in improved therapies for cancer patients. In recent years, 3D cell cultures were established as relevant preclinical cancer models suitable for high-throughput screening. However, their ability to represent physiological processes can still be significantly improved.

In this regard, we have combined three recent technological advances to develop a tumour organoid-on-chip device (TumOC): We integrated i) patient-derived 3D cell cultures (organoids) from colorectal carcinomas that recapitulate the architecture and heterogeneity of the original tumour, ii) oxygen micro sensor particles to assess cell viability in real time and iii) microfluidic perfusion for defined exposure of cytostatic drugs. We investigated colorectal cancer organoids with different cytostatic drug sensitivities in TumOC and compared the kinetic measurements with the endpoint measurements performed with an ATP assay.

With this setup, we can analyse the proliferation and proliferation-inhibiting, cytotoxic or regenerative effects in a time-resolved manner. Combined with the ability to mimic the rhythm of drug administration through microfluidic control of the cell environment, the mode of action and efficacy of drugs can be more accurately predicted.

Acknowledgments

This project is funded by the German Federal Ministry of Education and Research (BMBF), grant no. 161L0280A and 031B0763B.
Single nuclei RNA-seq profiling of primary human trophoblast organoids

Sunhild Hartmann\textsuperscript{a,b,c,d,e,f}, Ali Kerim Secener\textsuperscript{g}, Sebastian Mackowiak\textsuperscript{d}, Daniela Sofia Valdes\textsuperscript{a,d,e,g}, Theresa Zucker\textsuperscript{e}, Ruth Schmidt-Ullrich\textsuperscript{e}, Berthold Huppertz\textsuperscript{e}, Christina Stern\textsuperscript{a}, Florian Herse\textsuperscript{a,d,e}, Martin Gauster\textsuperscript{e}, Sabrina Geisberger\textsuperscript{e}, Naveed Ishaque\textsuperscript{k}, Ralf Dechend\textsuperscript{a,d,e,g,h}, Olivia Nonn\textsuperscript{a,d,e,g,k}

\textsuperscript{a} Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
\textsuperscript{b} Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia
\textsuperscript{c} Translational Obstetrics Group, The Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, Heidelberg, Victoria 3084, Australia
\textsuperscript{d} Experimental and Clinical Research Center, a cooperation between the Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association and the Charité – Universitätsmedizin Berlin, Berlin, Germany
\textsuperscript{e} Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
\textsuperscript{f} Institute for Medical Systems Biology (BIMSB), Berlin, Germany
\textsuperscript{g} DZHK (German Center for Cardiovascular Research), partner site Berlin, Germany
\textsuperscript{h} HELIOS Clinic, Department of Cardiology and Nephrology, Berlin, Germany
\textsuperscript{i} Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria
\textsuperscript{j} Department of Obstetrics and Gynaecology, University Hospital Graz, Medical University Graz, Austria
\textsuperscript{k} Berlin Institute of Health (BIIH) @ Charité

Introduction
Preeclampsia, a pregnancy-related complication, is a major cause of maternal and fetal morbidity and mortality. Currently, there are no precise methods to predict, prevent, or treat the disease. Multi-omics methods such as single-cell RNA-sequencing or spatial transcriptomics and proteomics can be used to study and understand preeclampsia pathophysiology. These methods enable the identification of pathways contributing to preeclampsia. We established trophoblast organoids and validated their potential to investigate these placental pathways.

Methods
Primary human trophoblast stem cells derived from first trimester placenta were used to generate trophoblast organoids as a model for cytotrophoblasts and syncytiotrophoblasts. Human trophoblast organoids were single nuclei sequenced and analysed according to previously annotated human placenta snRNA-seq data from a clinical cohort. Additional scRNA-seq organoid data from Shannon et al. [2] was analysed to investigate the distribution of metabolic markers in different cell types.

Results
All expected trophoblast cell identities could be replicated, and further validations of the trophoblast characteristics in human trophoblast organoids were conducted.

Literature
Application of High Content Imaging to Investigate Cell Fate Decisions in Human Brain Organoids Subjected to OGD

Lina Hellwig1,2, Harald Stachelscheid3, Philipp Mergenthaler1,2,3,4

1 Charité – Universitätsmedizin Berlin, Dept. of Neurology with Experimental Neurology, Charitéplatz 1, 10117 Berlin, Germany
2 Charité – Universitätsmedizin Berlin, Center for Stroke Research Berlin, Charitéplatz 1, 10117 Berlin, Germany
3 Charité – Universitätsmedizin Berlin, NeuroCure Clinical Research Center, Charitéplatz 1, 10117 Berlin, Germany
4 Acute Stroke Programme, Radcliffe Department of Medicine, University of Oxford, OX3 9DU, Oxford, UK
5 BCRT & Institute of Medical Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany

Ischemic stroke is one of the main reasons for acute neurodegeneration and holds massive morbidity and mortality for patients. Even though cells in the ischemic core die due to necrosis, neuronal cell death in the penumbra occurs via different pathways including apoptosis. This form of acute neurodegeneration can last from hours to days after the initial insult. To understand the mechanisms of apoptosis leading to acute neurodegeneration after ischemia, we derived human brain organoids (HBOs) derived from human induced pluripotent stem cells (hiPSCs) from oxygen and glucose, an established model of stroke. The oxygen and glucose deprived (OGD) hiPSC-HBOs were fixed, embedded and cryo-sectioned, and apoptotic activity quantified. To quantify the apoptotic activity of OGD treated hiPSC-HBOs, we established an automated high content imaging (HCI) pipeline consisting of the three main steps of HCI: (1) image acquisition, (2) image processing, and (3) image analysis. Application of automated image analysis algorithms allows a multiparametric data analysis, which gives information about a plethora of specific properties on a cellular or subcellular level. Based on these properties, possible differences between apoptotic and non-apoptotic cells can be identified and further classified in secondary analyses. Gaining a better understanding of why some cells are more prone to undergo apoptosis upon ischemia allows for more specific therapeutic strategies to prevent cell death after stroke.
In vitro models to study bacterial pneumonia

Karen Hoffmann¹, Chantal Weißfuß¹, Cengiz Goekeri¹, Christian M. Zobel², Martin Witzenrath¹, Geraldine Nouailles¹

¹. Charité - Universitätsmedizin Berlin, Berlin, Germany
². Department of Internal Medicine, Bundeswehrkrankenhaus Berlin, Berlin, Germany
³. German Center for Lung Research (DZL), Berlin, Germany

Our research focuses on studying the mechanisms and new therapeutic approaches of bacterial-caused pneumonia. To better comprehend the primary defense mechanisms against pulmonary pathogenic insults and avoid species particularities, a significant setback of animal models, we aim to work with advanced human cell models in vitro that emulate in vivo phenotypes.

On the one hand, we use human apical-out airway organoids and a human neutrophil cell line, HL-60, to study the effect of the immune system under phage therapy in Pseudomonas aeruginosa infection and elucidate possible synergistic mechanisms. While we know from in vivo studies that neutrophils are vital for clearing (phage-)resistant bacteria, it remains unclear whether this is a direct effect of neutrophil effector function or an indirect effect through activation of other immune effectors. With this in vitro model, we want to shed light on the interplay between epithelial cell infection, phage-induced bacterial killing, and neutrophil activation.

On the other hand, to study lung barrier function upon infection, we use electrical-cell substrate impedance sensing (ECIS) and a human lung-on-a-chip model that integrates physiological functions, including breathing motion and perfusion, by applying human primary epithelial as well as endothelial cells. By doing so, we revealed that modulation of the alveolar glycocalyx by treatment with Heparinase led to the breakdown of the epithelial barrier. Besides, treatment of alveolar epithelial cells with Heparinase resulted in an enhanced barrier failure upon infection with Streptococcus pneumoniae, reflecting the findings of in vivo studies.

Overall, our results show that advanced human cell models, which complement animal experiments by avoiding species particularities, are valuable tools for studying the therapy and mechanics of bacterial pneumonia in vitro.
Contribution of human organ models to COVID-19 research. A large-scale systematic review

*Maren Hülsemann*, Silke Kniffert*, Andreas C. Hocke and members of the EC3R and NUM OrganoStrat

1 Berlin Institute of Health at Charité (BIH), BIH QUEST Center for Responsible Research, Berlin, Germany,
2 Charité - Universitätsmedizin Berlin, Department of Infectious Diseases and Respiratory Medicine, Berlin, Germany

*contributed equally

Introduction

Organ models represent promising new approaches for studying human diseases and designing treatments. However, proving the reliability and robustness of these novel model types will be crucial for their broad acceptance as alternatives to certain animal models and in preclinical research.

The purpose of a systematic review is to systematically gather, evaluate, and synthesize existing evidence from a range of studies based on predefined inclusion and exclusion criteria. This methodology is well-established in clinical and preclinical animal research but is so far uncommon in in vitro research.

Objective

This study conducts a large-scale systematic review, to provide a rigorous and unbiased summary of the available evidence generated by studies utilizing human organ models. We are synthesizing evidence from 289 studies reporting the application of human organ models to study SARS-CoV-2. Our analysis focuses on infection studies and host factor expression experiments. We examine various types of organ models and their respective organ compartments, the assays conducted, their outcomes, and the quality of reporting. Our team of 24 reviewers, primarily organ model experts, systematically extracted information to assess the scientific outcomes and evaluate the overall quality of reporting of the included studies.

Results

We are currently synthesizing a comprehensive dataset and will present preliminary findings, emphasizing the various focus areas in the studies, as well as the frequency of applied assays, virus strains, detected host factors, etc. We will also deliver insights in the identified gaps in information provided in scientific publications that utilize human organ models. This data set can serve as a first use case to provide evidence-based information on improving reporting practices for human organ models. Our pilot work can offer practical insights to tailor systematic review methodologies to this emerging body of literature.
Utilizing CRC patient-derived 3D models to explore drug responses post KRAS inhibitor resistance

Tamara Isermann1,2, Annbalou Hasenburg1, Annabel Alig1, Sebastian Stintzing3, Christine Sers1,2, Björn Papke1,2

2. Charité - Universitätsmedizin Berlin, Institute of Pathology, Laboratory of Molecular Tumor Pathology and Systems Biology, Berlin, Germany
3. German Cancer Consortium (DKTK), Partner Site Berlin, German Cancer Research Center (DKFZ), Heidelberg, Germany.
4. Charité – Universitätsmedizin Berlin, Department of Hematology, Oncology and Tumorimmunology, Berlin, Germany.

Colorectal cancer (CRC) is currently the third most common cancer type and the second leading cause of cancer deaths in the world. KRAS mutations are observed in approximately 43% of CRC cases, with 66% of these being mutations in codon G12. Hence, inhibitors targeting KRAS\(^{G12}\) have gained increasing research interest in the past decade. Since the clinical approval of KRAS\(^{G12C}\) inhibitors in 2021, numerous patients have benefited from mutation-specific therapy. Unfortunately, after an average of 6 months treatment resistance is observed.

To better understand resistance mechanisms and identify potential vulnerabilities, we are establishing patient-derived organoids (PDOs) obtained from KRAS\(^{G12C}\) inhibitor treated CRC patients by fine-needle biopsies of liver metastases. Whenever possible, biopsies were obtained at various time points throughout the treatment. Once established, PDOs are treated with a small panel of EMA-approved drugs and their response is monitored.

Here, we present the data of one patient who developed a resistance to sotorasib, was then treated with multiple chemotherapies and rechallenged with sotorasib. Our PDOs mimic the patient’s drug response. The PDOs, like the patient, strongly responded to the chemotherapeutic agents Irinotecan and 5-FU, and poorly to Oxaliplatin and Lenvatinib. The KRAS\(^{G12C}\) inhibitor resistance due to a KRAS amplification was also observed in the PDOs.

To overcome this KRAS amplification-driven KRAS inhibitor resistance, multi-RAS inhibitors are currently undergoing clinical trials. The PDOs respond strongly to the multi-RAS inhibitor RMC-7977, suggesting patients with KRAS amplification could benefit from multi-RAS inhibitor treatment. We are currently optimizing the organoid establishment to drug treatment time and are extending our EMA-approved drug library to advance personalized therapies and thereby improve therapy outcome.

In conclusion, our CRC-derived PDOs mimic patient drug response to a high degree and their regular use in pre-testing personalized medicine should be considered. In the future we will monitor development of RAS inhibitor resistance by analyzing cell-free DNA.
Brain organoid technologies to model human brain development

Nicolai Kastelic, Ivanna Kupryianchyk-Schultz, Josephine Coburn, Agnieszka Rybak-Wolf

Organoid Platform, Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin

Understanding how the human brain functions in health and disease is one of the greatest challenges of modern science. Limited availability of human samples and ethical limitations hinder the study of the brain with methods from genomics and genetics. Recently, three-dimensional human brain organoids have emerged as a cutting-edge, genetically-tractable experimental system to study human brain development and function in vitro while retaining the cellular complexity and functionality of the fetal brain. There have been recently various approaches introduced to optimized brain organoid culture.

We will give an overview of current developments in Organoid Platform including a) different approaches for organoids vascularization and microglia incorporation b) generation of aged organoids c) organoid clearing and active labeling, d) optimization of bioreactor culture.
Unraveling causes of drug resistance in BRAFV600E CRC using single-cell mRNA sequencing of preclinical models

Anna Kotarac1,2,3,4, Alexander Malt5, Alexandra Trinks6, Hiroki Osumi7, Annalisa Lorenzato8, Federica Di Nicolantonio9,9, Mariangela Russo4, Ryoji Yao10, Naveed Ishaque5, Markus Morkel2,3,6,11, Alberto Bardelli8,12, Ulrich Keilholz1,2,3, Christine Sers2,3,11, Sebastian Stintzing2,3,4, Loredana Vecchione1,2,3,4,13

1. Charité Comprehensive Cancer Center, Charité-Universitätsmedizin Berlin, Berlin, Germany
2. German Cancer Consortium, partner site Berlin, Germany
3. German Cancer Research Center, Heidelberg, Germany
4. Department of Hematology, Oncology, and Cancer Immunology, Campus Charité Mitte, Charité -Universitätsmedizin Berlin, Berlin, Germany
5. Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Center of Digital Health, Berlin, Germany
6. Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Bioportal Single Cells, Berlin, Germany
7. Department of Gastroenterology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan
8. Department of Oncology, University of Turin, Italy
9. Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy
10. Department of Cell Biology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo,Japan
11. Department of Pathology, Charité - Universitätsmedizin Berlin, Berlin, Germany
12. IFOM ETS, The AIRC Institute of Molecular Oncology, Milano, Italy
13. Berlin Institute of Health at Charité - Universitätsmedizin Berlin,BIH Biomedical Innovation Academy, BIH Charité (Junior) (Digital) Clinician Scientist Program, Charitéplatz 1, 10117 Berlin, Germany

Chemotherapy remains the mainstay of palliative treatments for BRAFV600E metastatic colorectal cancer, with limited efficacy (1). Despite the approval of a new chemo-free regimen after systematic therapy, response is still poor, thus highlighting an unmet clinical need (2,3). Therefore, we aim to use single-cell mRNA sequencing (scRNAseq) to identify transcriptional subpopulations as biomarkers of resistance to conventional therapy. Nine BRAFV600E CRC cell lines and ten BRAFV600E CRC patient-derived organoids (PDOs) were treated with the following drugs in short term proliferation assays: erlotinib (OSI-744), encorafenib (LGX818), 5-flurouracil (5-FU), irinotecan (SN-38), oxaliplatin (L-OHP), OSI-744+LGX818, OSI-744+SN-38, 5-FU+SN-38, 5-FU+L-OHP, 5-FU+SN-38+L-OHP. Sensitivity was defined by IC50, AUC and Synergy Score. After isolation, cell lines and PDOs at baseline were subjected to barcoding, library preparation and sequencing (Illumina NovaSeq, 1600 mio. reads per library). Four out of ten PDO models and eight out of nine cell lines are sensitive to OSI-744+LGX818. This combination is additive and synergistic in all preclinical models. Additionally, these models are sensitive to 5-FU+SN-38 and 5-FU+SN-38+L-OHP, except for one model. Four out of nineteen tested models are resistant to 5-FU+L-OHP. While the combination of 5-FU+L-OHP and 5-FU+SN-38+L-OHP is rather additive and antagonistic, 5-FU+SN-38 is rather additive in our models. scRNAseq analysis is currently ongoing. Based on the results, we will further plan scRNAseq after drug perturbation. Furthermore, our scRNAseq analysis will be validated in publicly available and in-house clinical data sets (Fire 4.5 trial). This will help identify genomic determinants of BRAFV600E heterogeneity and resistance to conventional therapies.

References:
1. PMID: 27189416
2. PMID: 33503393
3. PMID: 32047001
Developing a humanized astrocytic calcium imaging pipeline for compound screening

Jeremy Krohn1,2, Camin Dean1,2

1. German Center for Neurodegenerative Diseases (DZNE) Berlin
2. Charité - Universitätsmedizin Berlin, Berlin, Germany

Summary:
Astrocytes are crucial mediators of diverse aspects of brain function such as energy metabolism and synapse formation and maturation. They form a vast network in the brain and signal both with each other and with neurons. Calcium is the primary information carrier in astrocytes and can be measured using fluorescent indicators.

Therapeutic approaches for cognitive diseases are commonly designed to influence neurons, but neglect astrocytes, despite them accounting for half the mass of the brain. Furthermore, screenings in murine cells result in low confidence of potential drugs that translate to humans.

Here we use astrocytic calcium signals and recent advances in analysis to develop an astrocytic compound screening pipeline in a humanized model.

Astrocytic calcium signals were measured using virally expressed calcium sensors based on GCaMP. Benchmarking was performed with a set of compounds of known effect in mouse hippocampal neuron-glia cultures.

We then established an induced pluripotent stem cell-derived human astrocyte culture system and again measured calcium signals. Human induced astrocytes showed wave-like events similar to those seen in mouse cells, and stimulation with ATP caused an increase of calcium events as expected. These preliminary data demonstrate that our humanized astrocyte cultures could replace murine cells in screens of compounds that affect astrocytic function.

In the future, we will validate the pipeline with compounds of unknown effect such as LSD. Calcium imaging data from this humanized model can be used to investigate potential drug effects on neuronal and astrocytic function and predict efficacy in treating cognitive diseases with greater confidence.
Characterization of resistance mechanisms and derivation of therapeutic approaches using organoids in cholangiocarcinoma

Anna Kühn*, Christopher Neumann*, Katharina Wansch1, Florian Dölvers1, Mihnea-Paul Dragomir2, Jana Ihlow2, Gregor Dauw3, Sebastian Stintzing1, Ulrich Keilholz4, Uwe Pelzer1
*both authors contributed equally

1. Department of Hematology, Oncology and Cancer Immunology, Charité Campus Mitte, Universitätsmedizin Berlin
2. Department of Pathology, Charité Campus Mitte, Universitätsmedizin Berlin
3. Department of Urology and Pediatric Urology, University Medical Center Johannes Gutenberg University, Langenbeckstraße 1, 55131 Mainz, Germany
4. Charité Comprehensive Cancer Center, Charité Universitätsmedizin Berlin

Cholangiocarcinoma (CCC) can be classified into two subtypes, intrahepatic and extrahepatic, based on its anatomical localization. It is a rapidly progressing tumor, resulting in the majority of patients being diagnosed at an advanced stage. Moreover, the high recurrence rate of up to 70% within the first 5 years after resection and the limited availability of effective therapies contribute to the low 5-year survival rate of 10-20%. In recent years, molecular inhibitors have shown promising results. However, 60% of patients do not exhibit genetically targetable alterations. For these patients, no biomarker- or platform-based methods for predicting treatment response exist. The objective of this study is to explore the potential of patient-derived organoid models (PDO) in predicting therapy outcomes, to elucidate the mechanisms behind resistance development, and to identify novel therapeutic approaches.

In the context of this project, 25 PDOs will be established from surgical specimens obtained from both treatment-naïve and pre-treated patients. The PDOs as well as the corresponding FFPE-tissues are subjected to histological, immunohistochemical and genetic characterization. Subsequently, the response of the PDOs to clinically relevant single agents, chemotherapy regimens and, in the presence of targetable genetic alterations, to molecular inhibitors is evaluated and compared with the clinical response of the patients. In a further step, secondary resistance to the gemcitabine/cisplatin combination therapy, which remains a main component of first-line therapy in advanced stages, is induced. Analogous to the clinic, this is achieved by cyclic exposure of the PDOs to the therapeutic agents in pharmacokinetically calculated tissue concentrations. Transcriptomic and proteomic analyses will be conducted before and after the induction to investigate the development of resistance mechanisms and to identify new, potentially targetable signaling pathways. Finally, the identified upregulated pathways will be therapeutically inhibited in order to derive novel therapeutic approaches for patients with CCC.
Elucidating SYNGAP1 Isoform Functions in Human Neurodevelopment Using Cerebral Organoids

Ivanna Kupryianchyk-Schultz¹, Daniel Bauersachs², Ralf Kühn², Manuel Irimia³, Sarah Shoichet¹ and Agnieszka Rybak-Wolf¹

1. MDC, Organoid Platform
2. MDC, Transgenics Platform
3. MDC, BIMSB
4. Charité - Universitätsmedizin Berlin, Berlin, Germany

SYNGAP1, encoding Ras/Rap GTPase-activating protein, is a critical gene involved in synaptic signaling and neurodevelopment. Mutations in SYNGAP1 are associated with intellectual disability and autism spectrum disorder (ASD). However, the specific functions of its multiple isoforms, generated by alternative splicing and transcription start sites, remain poorly understood. Current methods for studying SYNGAP1 functions rely on animal models, which do not fully recapitulate human neurodevelopment.

This project aims to elucidate the roles of different SYNGAP1 isoforms using human cerebral organoids as an alternative to animal models. Cerebral organoids, derived from pluripotent stem cells, offer a promising 3R approach by mimicking the human brain architecture and functionality. We will employ cutting-edge techniques including single-cell RNA sequencing, CRISPR-based gene perturbation, and BaseScope in situ hybridization to comprehensively profile SYNGAP1 isoform expression, manipulate their levels, and visualize their spatiotemporal distribution across different developmental timepoints: 15, 30, 60 days, roughly equivalent to 4, 10, 18 post-conceptional weeks of human development in vivo.

By developing this innovative 3D model system, we aim to uncover isoform-specific roles of SYNGAP1 in human brain development. Our approach will not only advance mechanistic understanding of SYNGAP1 biology, but also exemplify how cerebral organoids can serve as a powerful alternative to animal use. This project showcases the potential of human cerebral organoids to replace animal experiments in studying neurodevelopmental disorders, aligning with the 3R principles (Replacement, Reduction, and Refinement). We will provide updates on the progress of this work.
Protocol for cancer cell purification during PD3D model development

Jürgen Loskutov, Lena Wedeken, Barbara Seller, Cynthia Yapto, Rica Sauer, Manuela Regenbrecht, Christian Regenbrecht

1. CELLphenomics GmbH
2. Medizinisches Versorgungszentrum am Helios Klinikum Emil von Behring
3. ASC Oncology GmbH
4. DRK Kliniken Berlin, Clinic for Internal Medicine - Pneumology and Sleep Medicine
5. Institut für Pathologie, Universitätsklinikum Göttingen

Recently complex patient derived 3D (PD3D) cell culture models were recognized as a key tool for improvement in drug development, patient care and academic endeavor. However, the quality criteria, defining the “established” 3D culture, are not uniform between different groups. For instance, some groups view culture as established, when passage 3 is reached and misleading claims can undermine the whole field.

Surely, more physiological 3D cell culture models become, the more surprising challenges arise. Over the last couple of years, it was recognized that some non-cancerous cell populations tend to survive, strive and in some cases overgrow cancer cells in culture. This can significantly affect the success rate of PD3D model establishment and distort the subsequent drug screening results. We established two protocols for cancer cell purification during PD3D model development of non-small cell lung cancer, a tumor entity known to be notoriously difficult to obtain pure cancer cell cultures. Both protocols yield pure cancer PD3D models, recapitulating pathological characteristics of parental tumors. In some cases, both protocols yield very similar PD3D models, however, in others, cancer cell populations within PD3D models obtained through different protocols vary significantly. It is not yet clear what can cause such heterogenic response and further research is required to understand the underlaying phenomenon.

In the advent of functional precision oncology, our findings warrant strong collaborative ties including non-academic partners to ensure best-possible take-rates, quality and results not only from the research perspective, but also ethical. We owe this to all patients who donate their tissues for advancing research in this field.
Matrix-Free Human Organoid Monolayers Recapitulate Duodenal Barrier and Transport Properties

Kopano Valerie Masete1, Alain S. Massarani2, Dorothee Günzel2, Jörg-Dieter Schulze2, Hans-Jörg Epple1,3 and Nina A. Hering4

1 Department of Gastroenterology, Rheumatology and Infectious Diseases, Campus Benjamin Franklin Charité - Universitätsmedizin Berlin, Berlin, Germany
2 Clinical Physiology/Nutritional Medicine, Medical Department, Division of Gastroenterology, Infectiology and Rheumatology, Campus Benjamin Franklin Charité - Universitätsmedizin Berlin, Berlin, Germany
3 Antibiotic Stewardship Team, Medical Directorate, Campus Benjamin Franklin Charité - Universitätsmedizin Berlin, Berlin, Germany
4 Department of General and Visceral Surgery, Campus Benjamin Franklin Charité - Universitätsmedizin Berlin, Berlin, Germany

Transformed cell lines have been the gold standard to study epithelial barrier and transport function but as they are cancerous and derive from single individuals, they do not accurately recapitulate the intestine. Being a better representation of the intestine, intestinal 3D organoids are often digested and seeded in 2D as organoid monolayers on gelatinous matrix pre-coated surfaces for anchorage. As this coat could potentially affect the epithelial barrier and limit transport, we optimized a protocol to generate robust organoid monolayers that do not need a gelatinous matrix for anchorage. Using human duodenum-derived organoids from four healthy individuals, we characterized organoid monolayers seeded on Transwells phenotypically regarding polarization, tight junction formation and cellular composition, and functionally regarding uptake of nutrients, ion transport and cytokine-induced macromolecular transport. The organoid monolayers phenotypically recapitulated the duodenum with their transepithelial resistance dynamics and cell marker expression reflecting conserved patient-to-patient heterogeneity. Sub-lethal dose of tumour necrosis factor-alpha disrupted their barrier integrity, causing paracellular transport of 4 kDa Dextran and transcytosis of 44 kDa horseradish peroxidase. Functions of the duodenum such as glucose and short-chain fatty acid uptake were also conserved in these monolayers. Importantly, the common practice of seeding organoid monolayers on a layer of gelatinous matrix like basement membrane extract consistently hindered the transepithelial transport of chloride ions in response to forskolin. Hence, our matrix-free organoid monolayers can be used to study transcytosis, transcellular and paracellular transport of micro- and/or macromolecules and represent an improved epithelial barrier and transport model. As basement membrane extract is extracted from mouse tumours, our model furthers efforts to make organoid culture more animal-free, in line with the 3Rs Principle.
Developing humanized murine chronic brain slices as a 3D model of neurodegeneration

Muinjonov B\textsuperscript{1, 2}, Anca Margineanu\textsuperscript{2}, Séverine Kunz\textsuperscript{2}, Dr. René Jüttner\textsuperscript{2}, Siffrin V\textsuperscript{1, 2}

1. Charité- university Medical Center Berlin, 10117 Berlin, Germany
2. Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, 13125 Berlin, Germany

**Background.** Neurodegeneration is a progressive atrophy of neurons, which is present in neurodegenerative diseases. Up to date rodent models of studying neurodegeneration have been the most commonly used to investigate neurodegeneration in central nervous system (CNS). However, it has become more evident that these models may not fully recapitulate human disease as human and rodent astrocytes differ considerably in morphology and functionality. Our main hypothesis is that human astrocytes could be used to develop humanized chronic chimeric brain slices reduces species related discrepancies of current preclinical neurodegeneration models.

**Methods and preliminary results.** To investigate this, we developed organotypic chimeric humanized brain slices by transplanting human astrocytes differentiated from human induced pluripotent stem cells (hiPSCs after depletion of endogenous mouse astrocytes via diphtheria toxin expression under control of the Tamoxifen-inducible Aldh1l1 promoter. In parallel, we generated astrocyte progenitors from hiPSCs. Subsequently xenografted hiPSC-derived astrocyte progenitors differentiated into astrocytes that integrated morphologically within the mouse host hippocampal slices. We characterized morphology and distribution of these astrocytes in the brain slices. We found that hiPSCs astrocytes took on the typical morphology of CNS tissue-resident astrocytes and integrate robustly into their environment after the engraftment into the depleted slices in contrast with slices without glial depletion.

**Conclusion.** Developing chimeric brain slices by on partial depletion of native murine astrocytes, and transplanting human-derived astroglial cells into the mouse hippocampal slices to might be a promising method study individual human cells in an organotypic context. Establishing chimeric slices might paint a more detailed picture into the pathophysiology of different diseases and pinpoint the main culprit cellular interplay cascades, that initiate degeneration. These chimeric slices might be exploited to visualize complex interplay of different CNS cell populations or even intact neuronal network activity in an organ-like context.
Rabep2 as an adaptable driver of vascular homeostasis

Tomasz Nawara, Jan Kuom, Irene Hollfinger, Katja Meier, Jennifer Paech, Julia Kraxner, Emir Akmeric, Holger Gerhardt

Integrative Vascular Biology Laboratory, Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany.

Stroke is the second leading cause of death. The majority of stroke cases result from vascular occlusions, which lead to tissue ischemia and necrosis. Notably, the formation of a potentially fatal infarct can be avoided by redirecting the blood flow to the affected area through collateral blood vessels. The extent of pial collaterals varies between patients. In mice, 80% of this variation links to the Rab GTPase-effector binding protein 2 (Rabep2). While modulation of Rabep2 activity holds great therapeutic potential, it remains unknown why Rabep2 is exclusively needed for collateral homeostasis. Here, to define the molecular and mechanistic principles of Rabep2 action, we used human coronary arterial, microvascular, and umbilical vein endothelial cells grown as monolayers or as 3-dimensional organoids on a chip. Upon exposure to laminar and pulsatile flows, we observed Rabep2 polarization in arterial monolayers. This polarization was not observed in cells grown under static or oscillatory flow. This data suggests that Rabep2 adapts to different flows. We observed Rabep2 colocalization with Ve-cadherin-positive intracellular vesicles. BIOID further confirmed this interaction. Similar polarization was observed when mEGFP-tagged Rabep2 was expressed in venous monolayers exposed to laminar flow. This polarization was decreased upon expression of the inactive human genetic Rabep2 variant (p.Arg543His). This suggests that the predicted Rab5 binding residue is critical for Rabep2 function in collaterals. To parse out whether Rabep2 is critical for collateral formation, maintenance, adaptation to retrograde flow, or increase in fluidic shear stress, we are developing collaterals on the chip. With further incorporation of flow within the chip, we plan to establish an ex vivo stroke-collateral model. This work will form a foundation for exploring Rabep2 as a therapeutic target. Stroke patients often suffer from subsequent strokes. Therefore, defining whether activation of Rabep2 can promote collateralization post-stroke is a critical step in developing secondary prophylaxis.
Generation of a human 3D bone model to mimic glucocorticoid-induced osteoporosis in vitro

Johannes Plank1,2, Moritz Pfeiffenberger1,2, Alexandra Damerau1,2, Timo Gaber1,2, Frank Buttgereit1,2

1. Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Rheumatology and Clinical Immunology, Berlin, Germany
2. Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), ein Institut der Leibniz-Gemeinschaft, Berlin, Germany

Osteoporosis is a bone disease characterized by low bone mass and changes in bone architecture, often leading to pain, fractures and reduced mobility in affected patients. Glucocorticoid-induced osteoporosis (GIOP) is known as the most important form of secondary osteoporosis. To set up a GIOP in vitro model, we established and characterized a human in vitro bone model, subsequently using methylprednisolone to induce GIOP and later treat the model with anti-osteoporotic drugs.

To provide the basic scaffold for the structure of the bone model, mesenchymal stromal cells (MSCs) were differentiated on β-TCP. Afterwards, human osteoclasts, differentiated from CD14+ monocytes, were added, and the models were treated with 10^(-6)M methylprednisolone to induce osteoporosis. As pharmacological treatment of GIOP, we used alendronic acid and denosumab. Bone forming and resorbing activity was monitored using μ-computed tomography (μ-CT), supernatant analysis, gene expression, scanning electron microscopy and immunofluorescence.

Results showed that a ratio of 10^4 osteoblasts and 5*10^3 osteoclasts works best. We furthermore analysed the supernatant and detected secretion of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), osteoprotegerine (OPG), matrixmetallopeptidase 9 (MMP-9), free phosphate, and calcium. Our results confirmed the functionality of our 3D model. Subsequently, we transferred our untreated model to the osteoporosis-simulating model by treating with methylprednisolone and could show a disruption of bone homeostasis in favour of osteoclast activity. If these models were treated with alendronic acid or denosumab, markers of bone metabolism (Ca^{2+}, ALP, bone volume) returned to pre-pathophysiological.

Ultimately, we obtained an in vitro 3D co-culture of osteoblasts and osteoclasts simulating human native bone capable of mimicking key aspects of GIOP in vitro via treatment with methylprednisolone. As a proof of concept, GIOP was treated with established antiresorptive drugs showing an increase of osteogenesis markers.
The guardian of our airways: investigating molecular mechanisms of airway mucus clearance under inflammation

Tihomir Rubil¹,², Greta Harnisch¹,², Anita Balázs¹,², Marcus Mall¹,²

1. Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Charité - Universitätsmedizin Berlin, Berlin, Germany
2. German Centre for Lung Research (DZL), associated partner, Berlin
3. Berlin Institute of Health (BIH) at Charité, Berlin, Germany

Background
The elimination of inhaled pathogens, irritants, and pollutants from the airways by mucociliary clearance (MCC) is an essential function of the airway defense system. Many chronic airway diseases, such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis, are characterized by impaired MCC and the development of mucus plugging. Whereas it is known that chronic inflammation is a hallmark of these diseases and causes maladaptation of MCC, the exact molecular mechanisms that orchestrate MCC under inflammation remain unexplored.

Primary human airway epithelial cultures differentiated at air-liquid interface (ALI) recapitulate the native conditions of respiratory epithelium and provide a highly relevant, organotypical model system to study MCC in health and disease.

Methods
We generated highly differentiated ALI cultures from nasal brushings of healthy individuals and treated them with either interleukin-1β/neuregulin-1β (IL-1β/NRG-1β) – mediators of neutrophilic inflammation, or interleukin-13 (IL-13) – mediator of type 2 inflammation in asthma – to assess the functional impact of inflammation on MCC, as well as molecular changes associated with inflammation.

We measured mucus secretion and hydration (mucin and percent solids content), ciliary beat frequency (CBF), and mucus transport velocity (MTV).

Additionally, we performed fluorescence-activated cell sorting (FACS) and obtained whole-cell lysates to compare proteome changes between individual treatment groups.

Results
IL-1β/NRG-1β increased mucin secretion (MUC5B) and percent solids content, and decreased CBF and MTV. Additionally, FACS analysis indicated that IL-1β/NRG-1β increased the mucus secretory cell count. In contrast, chronic presence of IL-13 did not affect mucin secretion, mucus percent solid content, or CBF, but enhanced and MTV. Results of the proteome analyses will be presented.

Conclusions
This study represents a novel, integrated approach that provides mechanistic insights into cellular and molecular mechanisms that coordinate MCC during airway inflammation, complies with the 3R principle, and will be leveraged for studies of therapeutic modulation of MCC in respiratory diseases.
A model to study human intestine epithelium response to probiotics

Pilar Samperio Ventayol¹,², Jesper Andreas Bording Strickertsson ³, Ulla Festersen ³, Funda Gerceker Demirel¹,², Afonso Vieira¹,², Mastura Neyazi⁴, Sina Bartfeld¹,²,⁴

¹. Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany.
³. Immunology and Cell Biology, Novonesis, Bagsvaerd, Denmark
⁴. Research Center for Infectious Diseases (ZINF)/institute for Molecular infection Biology (IMB), Julius Maximilian University of Wuerzburg, Wuerzburg, Germany

The benefits of probiotics on human health have been extensively demonstrated in the last two decades. Although many clinical studies linked specific strains with gut outcomes against inflammation, the mechanisms of action are difficult to resolve in human studies. Animal models or tumor-derived cell lines are typically used to demonstrate the effects on the intestine in a more precise mechanistic manner. However, both models present documented limitations: species-specific characteristics, and dampened innate immunity responses or lack of cell heterogeneity, respectively. To study probiotic strain-specific effects on human innate immune response, we use patient-derived intestinal organoids, which maintain the region specificity of the gastrointestinal tract, including the innate immune components, and the cell heterogeneity, including the presence of enterocytes and secretory cell lineages. By growing human organoids on monolayer and applying previously reported media compositions, we obtained an apically accessible intestinal epithelium composed of differentiated enterocytes and secretory cells. We standardized the growth conditions by using commercially available and serum-free media and validated our results between two different laboratories. We built thus a reproducible in vitro model to mimic the environment where the first interaction of probiotics with human intestinal cells occurs. We anticipate that the innate immune response differs along the maturation state of enterocytes, and highlight adult-stem cell organoids as useful model to study in vitro probiotic strain-specific effects on human innate immune response modulation and enhancement of the intestinal epithelial barrier function.
Establishing a vascularized organ model on HUMIMIC chips

Alexandra M. K. Scherer¹, Beren Atac Wagegg², Hendrik Erfurth²

1. Technische Universität Berlin, Berlin Germany
2. TissUse Berlin, Berlin Germany

The HUMIMIC platform was developed to mimic human physiology and could potentially be used to reduce and replace animal tests during safety and efficacy testing according to the 3R principle (Replace, Reduce, and Refine). Chips allow single or multiple organ models to be cultured in perfused compartments connected by microfluidic channels.

However, the organ models that are used in the chips, often lack blood vessels and capillary structures. Vascularization plays an important role to mimic e.g. immune cell migration, model stability. Thus we aim to establish a vascularized organ model using fibrin-collagen matrix. Sacrificial structures are utilized to mimic blood vessels and capillary inside the fibrin-collagen-matrix. They are coated in endothelial cells to establish the endothelium. An organ of choice will be adapted to vascularized matrix for long term culture.
A breathable multi-compartment lung-on-chip model to study the (patho)physiological relevance of biological hydrogels in dynamic conditions

Konrad Schmidt¹, Felix Goerke², Lorenz Latta³, Nicole Schneider-Daum¹, Claus-Michael Lehr¹, Andreas C. Hocke⁴, Florian Schmieder², Frank Sonntag², Sarah Hedtrich¹,4,5

1. Berlin Institute of Health at Charité University Hospital, Berlin, Germany
2. Fraunhofer Institute of Materials and Beam Technology IWS, Dresden, Germany
3. Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbruecken, Germany
4. Department of Infectious Diseases and Respiratory Medicine, Charité University Hospital, Berlin, Germany
5. The University of British Columbia, Faculty of Pharmaceutical Sciences, Vancouver, Canada

Biological hydrogels such as lung mucus or surfactants exert critical protective and homeostatic functions in the human lung epithelium. Abnormalities in their composition and secretion are associated with increased susceptibility to both acute and chronic lung diseases. As of today, human disease models that allow studying of the relevant interdependencies are largely missing resulting in knowledge gaps as to how biological hydrogels contribute to certain disease states [1].

To address this gap, we have developed a dynamic multi-compartment lung model that enables the cocultivation of human alveolar and bronchial epithelial tissue and facilitates physiological hydrogel expressions. A 3D printed spacer initially separates both compartments and facilitates independent differentiation. Thereby, the bronchial part shows a typical pseudostratified epithelium and the alveolar epithelium a flattened phenotype. The airway epithelial cells are cultivated on a flexible base membrane incorporating collagen I and elastin, major components of the lung extra cellular matrix. Applying moisturised and pressurized air in a cyclic mode on the apical side of the model applies ≤ 20% linear strain on airway epithelial cells, mimicking (patho)physiological conditions.

While primary human cell based bronchial tissue models robustly produce lung mucus, we assessed the ability of freshly isolated alveolar epithelial cells, alveolar organoids and commercially available primary alveolar cells to produce alveolar surfactant. Freshly isolated alveolar epithelial cells exhibited the most promising potential, exhibiting elevated expression of surfactant proteins B and C. Immunofluorescence staining of alveolar type II (responsible for surfactant production in alveolar epithelium) surface marker HTII-280 suggested a conserved population inside the air-liquid-interface (ALI) culture. Furthermore, high barrier integrity exceeding 1000 Ω*cm² and maintenance of physiological morphologies support their utility as a reliable alveolar model for ALI studies.

Toward a human stem cell-derived neuronal network for high-throughput cognitive drug screening: Characterizing functionality in hiPSC-derived neurons and brain organoids

Hana Sheldon 1,2,5, Agnieszka Rybak-Wolf 3,4, Surjo Soekadar 5, Camin Dean 1

1. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Berlin, Germany
2. Einstein Center for Neurosciences (ECN) Berlin, Germany
3. Max Delbrück Center (MDC) Berlin, Germany
4. Berlin Institute of Health (BIH) at Charité, Berlin, Germany
5. Charité - Universitätsmedizin Berlin, Berlin, Germany

hiPSC-derived neurons and brain organoids have provided humanized models that recapitulate healthy and pathological mechanisms of developing brains. However, these models have not been fully characterized or investigated in terms of synapse function and plasticity. We study memory mechanisms at molecular, cellular and synaptic levels in mouse. To model human physiology more closely and increase the translatability of our research we aim to characterize synapse and circuit strengthening and weakening in hiPSC-derived neurons and brain organoids. We are testing for the presence of pre- and post-synaptic proteins by immunostaining for Synaptophysin, Bassoon, PSD-95, and Homer, as well as glutamate receptor sub-types. We found that chemical LTP protocols such as glycine activate cell populations identified by the immediate early gene marker cFos – a marker of cellular activity – in DIV52 organoids, indicating that there are functional NMDA receptors. We are also testing neuronal activation in response to magnetic stimulation protocols that lead to synapse strengthening. We are now testing cell-type specific markers to characterize activated cellular populations. We aim to establish a standardized hiPSC-derived neuronal network that demonstrates synapse strengthening and weakening under different stimulatory conditions. This can be used for high-throughput screening of cognitive drugs and validation of non-invasive brain stimulation methods.
A human Bone/Bone-marrow-on-a-chip system for preclinical investigation of new therapeutic approaches for autosomal recessive osteopetrosis

Nina Stelzer1,2, Melanie-Jasmin Ort1,3,4, Martin Textor1,4, Ioanna Maria Dimitriou1,4, Lea Heinemann1,5, Luis Lauterbach1,5, Dario Gajewski5, Uwe Kornak5 and Sven Geißler1,3

1. Center for Regenerative Therapies (BCRT), Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany
2. Department of Medical Biotechnology, Technische Universität Berlin, Berlin, Germany
3. Julius Wolff Institute, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany
4. Institute of Chemistry and Biochemistry, Department of Biology, Chemistry and Pharmacy, Berlin, Germany
5. Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany
6. Berlin Center for Advanced Therapies (BECAT), Charité Universitätsmedizin Berlin, Berlin, Germany

This project introduces a human Bone/Bone-Marrow-on-a-Chip system to connect fundamental research and translational applications. Utilizing the HUMIMIC Chip2 platform from TissUse, our approach is based on a decellularized human bone scaffold and sequential colonization allowing for 6 weeks of long-term cultivation. Within this cultivation period, we observe the maintenance and self-organization of bone-forming cells, bone-resorbing cells and immune cells, which enable us to assess the dynamics of the bone matrix and immune responses within the bone marrow microenvironment.

In parallel, a system was developed utilizing induced pluripotent stem (iPS) cells. These cells offer the advantage that genetic diseases can be mimicked or repaired. Currently, our Bone-on-a-Chip system is being used to preclinically test two gene therapy strategies for targeting autosomal recessive osteopetrosis (ARO). ARO is a rare genetic disease characterized by impaired bone resorption, for which the only treatment available is allogeneic stem cell transplantation. Since this is a major risk for those affected, our system offers a new complete animal-free approach.

To validate the physiological relevance of our in vitro bone model, we currently undergo a single-nuclei RNA analysis. Additionally, fluorescence imaging is applied to provide a structural comparison between the in vitro and in vivo tissues. During long-term cultivation, the system is monitored by analyzing soluble factors and validating immune cell populations by fluorescence-activated cell sorting (FACS). Co-registration of the scaffold before and after cultivation allows for quantification of bone turnover, reflecting a potential imbalance between bone formation and resorption, as in the case of ARO.

In conclusion, our Bone-on-a-Chip system is a versatile translational research tool. Long-term culture allows us to observe orchestrated cell behavior, enabling detailed investigation of bone dynamics and immune responses. The integration of iPS cells increases the usefulness for the simulation of genetic diseases and enables applications in both basic and translational research.
Towards Immunity on Chip – Immune Cell Perfusion of an IPSC-derived Intestinal Model

Rosanna Stolberg¹, Anja Hellwig¹, Eva-Maria Dehne¹
1. TissUse GmbH, Oudenarder Straße 16, 13347 Berlin

Understanding the complexities of the human intestinal epithelium and its interaction with the immune system is crucial to advance biomedical research. In this context, the development of a microfluidic chip-based intestinal model derived from induced pluripotent stem cells (iPSCs) holds immense significance. This study aimed to create an innovative model to closely mimic the intestinal epithelium, assess its characteristics and functionality and integrate immune cells to simulate the microenvironment of Peyer's patches.

Following organoid differentiation, cells were dissociated and seeded onto transwells to establish a functional monolayer. Barrier integrity was evaluated via trans-epithelial electrical resistance (TEER) measurements and functional assays. To replicate Peyer's patches, a three-dimensional model was constructed, integrating a hydrogel between the epithelium and a leukocyte permissive membrane. B-cells were incorporated to simulate B-cell follicles within this 3D setup. Immunohistochemical characterization validated the similarity of the derived organoids to native intestinal epithelium. The monolayer demonstrated robust barrier integrity, confirmed by TEER measurements. The development of the 3D model successfully integrated B-cells within a simulated Peyer's patch microenvironment. Incorporation into a microphysiological system enabled immune cell migration from circulation into the model, facilitating interactions with the B-cells. This study presents the successful establishment of an immune-competent intestinal model derived from iPSCs. The model effectively replicates the native intestinal epithelium, exhibits barrier functionality, and recreates a specialized setup including simulated Peyer's patches. This advanced model holds promise for advancing our understanding of intestinal physiology, immune responses, and its potential applications in drug development and personalized medicine.

Funded by the European Union under Grant Agreement Nr. 101057438. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HADEA). Neither the European Union nor the granting authority can be held responsible for them.
Human Gastrointestinal Organoids to Model Innate Immune Response and Infection

Afonso Vieira\(^1\), Mastura Neyazi\(^1\), Pilar Samperio Ventayol\(^1\), Sina Bartfeld\(^1\)

1. Department of Medical Biotechnology, Technische Universität Berlin, 10623 Berlin, Germany; 2. Si-M/"Der Simulierte Mensch", a Science Framework of Technische Universität Berlin and Charité Universitätsmedizin Berlin, 10623 Berlin, Germany

Human intestinal organoids are structures derived from stem cells, isolated from intestinal crypts. These 3D structures not only have the potential to include most of the cell types present in the human intestine but also preserve a similar cellular organization with crypt-like structures populated with stem cells. Moreover, organoids can be easily used for imaging, are not immortalized, but can be expanded indefinitely, and are suitable for medium to large-scale experiments. Characteristics such as these make human intestinal organoids a useful model to study infection and host-pathogen interactions.

In our lab, we study Enteropathogenic E. coli (EPEC) infection using human intestinal organoids as a host model. EPEC is a gram negative, rod-shaped bacterium which can infect the human gut. The attachment of the bacteria to the epithelial barrier of the intestine leads to translocation of virulence factors and the characteristic attaching/effacing lesions. EPEC is currently one of the big causative agents of diarrhea in the world and affects many children across the globe. One of the, so far unexplained, characteristics of EPEC is that it causes more severe symptoms in young children when compared to adults, where the infection is easily resolved. We aim to improve the understanding of what determines the different outcome of infection in adults and young children.
Light-inducible patterning of organoids: unraveling the role of WNT3A in human hippocampal development

Miriam Wandres1,3, Nele Kagelmacher1, Nicolai Kastelic, Denise Aigner1,3, Gwendolin Thomas1, Anastasiya Boltengagen1, Mara Fischer2, Andreas Hocke2, Giuliana Dube1, Lukas Fajß2, Poojashree Bhaskar1, Nikos Karaiskos1, Daniel Perinán1, Marie Schott1, Lieke van de Haar1, Agnieszka Rybak-Wolf1, Dietmar Schmitz2, Nikolaus Rajewsky1,3

1. Max Delbrück Center for Molecular Medicine in the Helmholtz Association; 2. Charité - Universitätsmedizin Berlin; 3. Humboldt-Universität zu Berlin

Brain organoid research is advancing our understanding of brain development by replicating specific brain regions. Morphogens play a pivotal role in orchestrating spatial and temporal patterning during embryonic brain development, guiding the emergence of specialized cell types and regional identities. Leveraging these signalling molecules in vitro facilitates the generation of brain organoids resembling distinct regions such as the hippocampus, essential for learning and memory. WNT morphogens, especially WNT3A, are crucial in hippocampal formation, guiding neural stem cell differentiation into excitatory and inhibitory neurons, thus shaping hippocampal circuitry. Here, we employ a light-inducible gene expression system to activate WNT3A in induced pluripotent stem cells and organoids. The overexpression of WNT3A in embryoid bodies triggers a striking phenotypic transformation in organoids, characterized by the emergence of distinct cell layers resembling hippocampal architecture. Notably, PROX1, a pan-hippocampal marker predominantly expressed in the dentate gyrus, is observed in a defined layer within light-stimulated organoid cells by day 30. Single-cell sequencing of these organoids confirms the expression of other hippocampal markers such as ZBTB20, GAD1, KA-1, NEUROD2, SPARC, and MEIS2. Spatial transcriptomics data produced with Open-ST further confirms the layered organization of these organoids. Beyond fusing WNT3A overexpressing organoids with cortical organoids to create multi-regional assembloids, we take advantage of the optogenetic system to spatially activate a ‘hippocampal-like organizer’ for studying cortical-hippocampal interactions. This method not only advances our comprehension of hippocampal brain organoid differentiation and cortical-hippocampal dynamics, but also presents promising avenues for therapeutic innovation in neurological disorders.
Identifying Mechanisms of chemotherapy resistance in pancreatic cancer using organoid models

* both authors contributed equally

1. Charite Campus Mitte, Freie Universität Berlin, Humboldt Universität zu Berlin, Berlin Institute of Health, Department of Hematology, Oncology and Cancer Immunology
2. Department of Pathology, Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt- Universität zu Berlin and Berlin Institute of Health, Germany
3. Charité Comprehensive Cancer Center, Charité Universitätsmedizin Berlin

Pancreatic cancer is one of the deadliest cancers, with an overall survival rate of only 10%. Effective treatment options are scarce because pancreatic cancer is highly resistant to chemotherapy. Currently, up to 80% of patients relapse within the first five years.

In recent years, organoid technology has emerged as a promising approach to assessing treatment response in PDAC. However, organoid models have only been used to assess drug response at a single time point. To improve our understanding, this project aims to develop a dynamic predictive platform and explore the evolving nature of chemotherapeutic resistance.

In the first phase of the project, we established 13 patient-derived organoid cultures from treatment-naive patients and characterized these PDOs by histology, immunohistochemistry, DNA panel sequencing and treatment response analysis. To induce resistance, 4 established cultures were subjected to multiple cycles of chemotherapy, closely mimicking the clinical conditions faced by patients. We will identify resistance mechanisms at a functional level by analyzing the transcriptome and proteome of patients before, during and after chemotherapy.

To investigate whether these mechanisms are present in a small subset of primary resistant cells before treatment or whether they develop in secondary resistant cells during treatment, we generated single cell-derived organoids and will perform transcriptomic and proteomic analyses on these organoids. This allows the identification of primary resistant clones within the heterogeneous, treatment-naive tumor.

In summary, our research project aims to identify mechanisms of chemotherapy resistance in pancreatic cancer by implementing transcriptomic and proteomic analyses. The identification of such mechanisms will facilitate the development of personalized therapeutic strategies. In the long term, high-throughput drug screening using dynamic predictive platforms may lead to significant advances in the treatment of pancreatic cancer.
Magdeburg patient- individual, visceral organoid bank – a multi organ approach modeling tumor, tumor microenvironment and control tissue in primary and secondary malformations

Thomas Wartmann\textsuperscript{1}, Roland S. Croner\textsuperscript{1,2} and Ulf D. Kahlert\textsuperscript{1,2}

1. Molecular and Experimental Surgery, University Clinic for General-, Visceral-, Vascular- and Transplantation Surgery, University Medicine Magdeburg (UMMD), Otto-von-Guericke University Magdeburg, Germany
2. An-Institute for Operative Medicine gGmbH, Otto-von-Guericke University Magdeburg, Germany

As a derivative of our bio banking efforts, we establish a human organoid platform. Compared to commercial CRO offers, our system qualifies with certain technological assets. This includes access to in depth multi-parameter clinical metadata of donors and their clinical course associated to the model systems; the availability of patient- and organ-matching non-tumor organoid systems; the availability of patient-matching components of the cellular microenvironment (stroma) or microenvironment (peripheral-immune cells) as well as a collection of model systems of patient-matching primary tumor and thereof derived secondary malignancies. Our lab is equipped with instrumental infrastructure allowing the state of the art functional assessments of cells as well genetic engineering. We are interested in understanding molecular mechanisms of therapy resistance and cellular invasion/metastasis. The embedment of our unit in clinical treatment center allows initiation investigator initiated and/or sponsored clinical trials, including the use of PDO for co-clinical testing.
A human iPSC-based platform for CRISPR-perturbations in liver systems

Julian Weihs1, Mijuna Meindl1, Calvin-Lee Classon1, Susanna Quach1, Katarzyna Ludwik2, Harald Stachelscheid2, Philip Butler1 Milad Rezvani 1, 3, 4, 5
1. Charité Universitätsmedizin Berlin, Department of Pediatrics, Division of Gastroenterology, Nephrology and Metabolic Medicine, Augustenburger Platz 1, 13353 Berlin
2. Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, BIH Core Unit pluripotent Stem Cells and Organoids (CUSCO), Berlin, Germany
3. Berlin Institute of Health, Clinician Scientist Program
4. Berlin Institute of Health, Center for Regenerative Therapies
5. Cincinnati Children’s Hospital Medical Center, Division of Gastroenterology, Hepatology and Nutrition, Cincinnati, OH 45229, USA.

Rodent models play a pivotal role in investigating genetic factors in complex organ systems such as the liver. Species differences, however, limit the translation of findings, underscoring the necessity for equivalent human systems. To overcome these limitations, we establish a human iPSC-based genetic perturbation platform using CRISPR-activation (CRISPRa) with a proof of concept in liver-based systems.

Specifically, we generated a doxycycline-inducible iPSC-CRISPRa line, by inserting a neomycin-resistant, TET-on dependent dCas9-VPR construct into the AAVS1 safe harbor. Thus, enabling temporally-controlled, guide-RNA (gRNA) -based perturbations in human models. After showcasing the functionality of the CRISPRa system in iPSC-stage, we validated its genomic and pluripotent integrity. We then evaluated its CRISPRa capabilities first in 2D Hepatocyte-like cells (HLCs) undergoing injury and then, more complex 3D liver-like organoids.

The CRISPRa-line efficiently differentiated into 2D HLCs, similar to its non-engineered counterpart. In HLCs undergoing cellular injury, the CRISPRa system specifically increased the expression of genes targeted by respective gRNAs. Also, targeting transcription factors using CRISPRa, resulted in further downstream effects of expression levels.

Assessing the CRISPRa impact in more complex models, we tested the overactivation of one hepatocyte transcription factor in the development of iPSC-derived multi-cellular liver organoids. These show in current protocols under-representation of hepatocyte-like cells. After inducing HNF4A in the early development, we saw hepatocyte-enriched organoids, based on gene expression. In addition, obstructive effects mediated by doxycycline in the control group were attenuated by HNF4A overactivation. Thus, we show functional perturbations of developing complex in vitro organoid models.

In summary, we here develop a universal hiPSC-based CRISPR platform, showcasing its potential in 2D and complex 3D liver-based models. Expanding this system to other cellular models will allow for assessing genetic perturbations in a wide range of developmental, physiological, and pathophysiological questions.
3D Bioprinted Perfusable Vascularized Organ Models via Sacrificial-Free Direct Ink Writing

Dongwei Wu1, Shumin Pang1, Johanna Berg1, Yikun Mei1, Ahmed S. M. Ali1, Viola Röhrs1, Beatrice Tolksdorf1, Judith Hagenbuchner2, Michael J. Ausserlechner2, Hedwig E. Deubzer3, Aleksander Gurlo1, Jens Kurreck1

1. Technische Universität Berlin, Berlin, Germany
2. Medical University Innsbruck, Innsbruck, Austria
3. Charité-Universitätsmedizin Berlin, Berlin, Germany

Perfusable organ models have seen broad applications in biomedical research, offering better insights into physiological responses with enhanced relevance compared to the statically cultured ones. To generate these organ models, 3D bioprinting has been widely used to fabricate various constructs with different structures. However, it has encountered many limitations, such as low efficiency, high cost and incompatibility with multiple materials. Therefore, this study focuses on developing a quick and economical bioprinting approach to fabricate in vitro 3D organ models with vasculature and perfusion features, which are crucial for mimicking physiological conditions. This method was termed as Sacrificial-Free Direct Ink Writing (SF-DIW), the printability of which was enhanced by optimizing the printing parameters through rheological measurement, extrusion test and cell viability assessment. Liver models with different cancer metastases were fabricated using this approach. The produced hepatic models after perfusion culture had higher cell viability and enhanced liver functions compared to those in static culture. It was shown that the perfusable liver model also supports the growth of different cancer spheroids in the hepatic environment. Furthermore, they were demonstrated to have the ability to evaluate the bioactivation and metabolism of a prodrug. Therefore, the liver models created through SF-DIW, featuring human origin, offer a syngeneic platform for studying cancer metastasis and drug responses. The successful bioactivation of ifosfamide in the liver model further underscores the physiological relevance of the generated models.
Establishing a cholangioid library for biliary-niche-on-a-chip multicellular models for the study of ductular reaction

Guo Yin¹, Lan Tian¹, Hanyang Liu¹, Natalia Martagón Calderón², Milad Rezvani², Frank Tacke¹, Adrien Guillot¹.

¹. Charité - Universitätsmedizin Berlin, Berlin, Germany
². Charité - Universitätsmedizin Berlin, Berlin, Germany; Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; Berlin Institute of Health, Berlin, Germany; Berlin Institute of Health (BIH) at Charité, Berlin, Germany

Background and Aims: Ductular reaction (DR), characterized by cholangiocyte proliferation, inflammation, and liver fibrosis, is a common hallmark of virtually all chronic liver diseases. Multiple cells are involved in DR. Therefore, we took advantage of a library of mouse intrahepatic cholangioids (mICO) generate from healthy and DR mouse models, and establish a biliary-niche-on-a-chip (BoC) as an in vitro perfusion multicellular system to study DR.

Methods: Intrahepatic cholangiocytes were isolated from wild-type (WT) and Mdr2-deficient (KO) mice by magnetic anti-CD146 microbeads and expanded to generate 3-dimensional organoids. Bulk RNA sequencing and multiplex immunofluorescence staining were performed to confirm cell identity. Dissociated WT and KO mICOs were seeded in the BoC together with mouse hepatic stellate cells, liver sinusoidal endothelial cells, and macrophages. The BoC was perfused with fresh blood circulating immune cells. Immunofluorescence staining was performed on the cells on the membrane in BoC, and the perfusate was collected for flow cytometry.

Results: WT and KO mICOs were successfully generated and expanded. Differences of proliferation, phenotypes, and cytokines between WT and KO mICOs were found by RNA sequencing. The results of multiplex immunofluorescence staining revealed a cluster of macrophages accumulating around biliary niche in KO mice liver. Immunostaining on the BoC membrane indicated successful harvest of multiple cells. And the results of flow cytometry revealed an increase accumulation of immune cells in the BoC.

Conclusion: mICOs and BoC which contained multiple cells were established for the in vitro study of DR. These multicellular models were a novel tool to study the crosstalk between different cell types in DR, reducing the number of experimental mice as well.
Defining inter- and intra-patient matrix heterogeneity of liver tissue and colorectal liver metastases


1. Department of Surgery, Charité - Universitätsmedizin Berlin, Berlin, Germany.
2. Institute of Pathology, Molecular Tumor Pathology, Charité – Universitätsmedizin Berlin,
# Co-authors
* Co-corresponding authors

Introduction:
Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide, accounting for approximately 900,000 deaths each year. Despite advances in multimodal therapy, recurrence remains a significant challenge with 30-50% of CRC patients developing colorectal liver metastases (CRLM) during their disease. Therefore, there is an urgent need for a deeper understanding of tumor biology and metastatic mechanisms. However, cell culture models that mimic metastatic behavior are limited.

Defining liver matrix compositions of 3D in vitro models:
To improve therapeutic strategies for CRLM, we developed an advanced 3D in vitro model using decellularized liver matrix (dLM) and CRLM matrix (dCRLM). Our model consists of the native liver extracellular matrix (ECM) composition and induces a metastatic phenotype of colorectal cancer cells.

To define inter- and intra-patient liver matrix heterogeneity, we isolated and decellularized liver matrix (dLM) and CRLM matrix (dCRLM) from 11 patients who underwent liver resection.

By qualitative mass spectrometry analysis, we identified 648 proteins of which 108 proteins were recognized as ECM components. We observed low inter-sample variability in the overall protein composition. Nevertheless, we identified 73 proteins with statistically significant differential abundance between normal non-cancerous liver and metastasis (Ration >1.2<0.8, P < 0.05), of which 15 with a fold change of >3 were consistently up- or downregulated within the ECM. Many of the proteins upregulated in the metastatic matrix have been previously implicated in metastatic progression, including FBN, COL5A3, POSTN, HSPD1, HSPA9.

Conclusion and outlook:
Our proteomic analysis of decellularized ECM scaffolds from CRLM and LM revealed significant differences in specific protein abundances, highlighting the tumor’s ability to tailor the tumor ECM to its metastatic needs. These differences suggest that specific ECM components in CRLM may influence tumor behavior. Our study highlights the potential of patient-derived 3D in vitro models for a deeper understanding of the metastatic process and the influence of the tumor microenvironment. By replicating the native tumor ECM and analyzing its proteomic intricacies, we provide a basis for precision medicine strategies for managing colorectal liver metastases. Next, to gain a better understanding of the cellular adaption process to the different matrix compositions, we will add a reference cancer organoid line to each individual dLM and dCRLM matrix.
Primate-specific miRNAs in the context of neurodevelopment

Denise Aigner¹, Miriam Wandres¹, Agnieszka Rybak-Wolf⁴, Nikolaus Rajewsky¹

1. Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine, Berlin, Germany

The brain is the result of a million-year long evolutionary process benchmarked by the appearance of the neocortex together with higher cognitive functions. The size of the neocortex distinguishes humans from other primates, this is associated with an increase of upper layer neurons and surface area. Scientific evidence suggests that one reason for the phenotypic changes between primates and other mammals are changes in the complexity of gene expression patterns. Important regulators of cell-type specific gene expression patterns are microRNAs (miRNAs), which are known to play a role in neurodevelopmental processes and are highly abundant in the brain. In this study we aim to investigate the expression and function of primate-specific miRNAs during neurodevelopment using human brain organoids as an experimental model. For this, we have implemented a reproducible dorsal cortical organoid protocol. miRNA analysis of developing cortical organoids revealed that previously described neuronal miRNAs highly correlated with the emergence of neuronal cell types. Although around 80 primate-specific miRNAs validated by MirGeneDB2.1, exist, only a few were detected in cortical organoids. These miRNAs exhibited a dynamic time-dependent expression profile. Next, we will analyse the cell-type specific expression of selected miRNAs using In situ hybridization. Moreover, with perturbation experiments we intend to assess the impact of selected primate-specific miRNAs during cortical development. Additionally, we aim to identify miRNA targets and their possible interactors in a cell-type specific manner.