

Epithelium in an intestinal assembloid © Manqian Lin & Michael Sigal

Human **3D ORGAN MODELS** NETWORKING EVENT

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ABSTRACTS

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1

First steps towards an *in vitro* fracture hematoma model on a chip

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Musculoskeletal disorders, particularly fracture healing impairments, represent a major global burden, contributing significantly to years of living with disability and socioeconomic costs. Approximately 10% of all fractures result in healing disorders, necessitating long-lasting and often insufficient therapeutic interventions. Despite promising preclinical animal studies, many innovative therapies have failed in clinical trials, highlighting the need for more predictive, human-relevant *in vitro* models. To address this, we have successfully optimized and scaled up a human-based 3D *in vitro* model mimicking the initial phase of fracture healing using organ-on-a-chip technology, thereby offering a sophisticated alternative to severe animal experimentation.

Building upon a previously established and validated 3D co-culture model of fracture hematoma (FH) and bone, we integrated our system into the Mimetas Organ-on-a-Chip platform. Here, we achieved robust perfusion using osteogenic induction medium with human platelet lysate, replacing FCS. The perfused environment induced key osteogenic and inflammatory signatures within 48 hours, closely resembling human fracture hematoma tissue.

Next, comparative analysis using flow cytometry, qPCR, and ELISA confirmed high concordance between the perfused chip model, our prior non-perfused model, and human *ex vivo* fracture hematoma samples, validating the biological relevance and reproducibility of the new system.

In the last step we introduced a significant advancement by incorporating HUVECs into the model via pre-coated channels and hydrogel-based embedding. This enabled the successful formation of early vascular-like structures – however not yet within the hematoma model –, recapitulating aspects of angiogenesis observed during the soft callus phase of human fracture healing.

In conclusion, our study demonstrates that this fully humanized, perfused, and partially vascularized 3D *in vitro* model accurately mimics early fracture healing events. It represents a scalable, high-throughput platform for studying bone regeneration and screening therapeutics, offering a powerful tool for translational research while reducing reliance on animal models.

2

SLC26A9 is important for coordinated transepithelial chloride transport and mucociliary clearance in healthy and cystic fibrosis airway epithelium

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Genetic studies identified solute carrier family 26 member 9 (SLC26A9) chloride transporter as a modifier of lung function in health and in cystic fibrosis (CF) lung disease and mouse studies showed that SLC26A9-mediated chloride secretion is required to prevent mucus obstruction in type 2 airway inflammation suggesting that SLC26A9 is a promising therapeutic target in CF and potentially in other muco-obstructive lung diseases. However, the role of SLC26A9 in coordinated transepithelial ion transport and mucociliary clearance in the human airway epithelium is not well understood.

We quantitatively assessed SLC26A9 function in primary nasal epithelial cultures from healthy donors and CF patients homozygous for the F508del-CFTR mutation, as well as CF patients homozygous for class I CFTR mutations. Transepithelial short circuit current measurements showed that SLC26A9 mediates ~30% of constitutive and cAMP-stimulated chloride transport, whereas inhibition of both SLC26A9 and CFTR abolished chloride transport in healthy cultures. Furthermore, treatment with specific SLC26A9 inhibitor S9-A13 decreased the mucociliary transport rates. In CF cultures with F508del-CFTR and class I CFTR mutations, residual cAMP-stimulated chloride transport was largely mediated by SLC26A9, whereas inhibition of SLC26A9 function strongly reduced mucociliary transport velocity.

Collectively, these results indicate that SLC26A9 is an important player in transepithelial anion secretion and for the maintenance of mucociliary clearance in the airways of healthy individuals as well as patients with CF, and support its role as potential therapeutic target in muco-obstructive lung diseases. Further, this study demonstrates the utility of highly differentiated primary human airway epithelial cultures to identify novel therapeutic targets in chronic lung diseases.

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3

Co-culture of patient-derived gastric cancer organoids and a liver model on a chip allows monitoring of patient-specific responses to prodrugs

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Patient-derived organoids are a promising tool for drug development and personalized therapies. However, they lack hepatic metabolism, limiting the testing of prodrugs, such as capecitabine, a derivative of 5-fluorouracil (5-FU), used in cancer treatment. Multi-organ chips model inter-organ communication. Here, we present co-culture of gastric cancer-derived organoids (PDGCO) with a liver model on a chip. We assessed capecitabine and 5-FU effects on PDGCOs prior and after hepatic biotransformation using viability, staining, gene expression, and metabolic analyses. Individual culture of PDGCOs or liver model on chip supported the growth of the PDGCOs and promoted differentiation of the hepatocytes, respectively. The co-culture of PDGCOs and the liver model allowed observation of patient-specific prodrug responses by demonstrating inter-individually different but intra-individually 5-FU-recapitulating capecitabine effects on PDGCO viability only if the liver model was present. Automated multi-organ chips could significantly enhance preclinical drug efficacy and metabolism testing, providing reliable data for future clinical trials.

4

Diet modulates colonic epithelial proinflammatory responses by influencing butyrate-producing microbiota.

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Obesity, a growing global health concern, is closely associated with numerous diseases and is influenced by dietary habits that can alter the gut microbiota. Emerging evidence indicates that dysbiosis - an imbalance in gut microbial communities - is linked to chronic inflammation, a process implicated in both inflammatory and malignant diseases throughout the body. However, the precise mechanistic connections between diet, gut dysbiosis, and inflammation remain incompletely understood. Our study hypothesized that high-fat diet (HFD)-induced changes in the gut microbiota could modify mucosal immune responses, increasing intestinal inflammation and elevating susceptibility to systemic inflammatory and malignant conditions. In experiments, mice fed an HFD for 13 weeks experienced a 67% increase in body weight. Analysis of colonic tissues using immunofluorescence revealed significant immune cell infiltration, particularly macrophages, as indicated by the marker Iba1. 16S rRNA sequencing of the microbiota in HFD-fed mice showed marked changes in microbial composition and diversity, including a reduction in butyrate-producing bacteria. Butyrate, a short-chain fatty acid with key roles in gut health as a nutrient for differentiated cells and a modulator of physiological functions, was found to be significantly reduced in stool samples of HFD-exposed mice, as confirmed by gas chromatography-mass spectrometry. In parallel experiments using human epithelial organoids, exposure to lipopolysaccharide (LPS) activated NF- κ B and increased expression of the proinflammatory chemokine IL-8. Pretreatment with butyrate attenuated this inflammatory response, demonstrating its ability to suppress proinflammatory epithelial signals. These results highlight butyrate's potential role in mediating tolerance to the gut microbiota and suggest that its depletion, driven by dietary changes, may promote heightened proinflammatory responses in the gut epithelium. Further research is ongoing to clarify the specific effects of butyrate on mucosal immunity and overall human health.

5

Helicobacter pylori infection causes gastric mucosal reprogramming into a proinflammatory, fetal-like state

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Helicobacter pylori (H.pylori) infection is the main risk factor for gastric cancer. Infection leads to chronic inflammation and gastric gland hyperplasia. We found that infection induces alterations in the stromal niche, such as inhibited secretion of BMP ligands and increased expression of BMP inhibitors. BMP signaling is known to be involved in differentiation of base progenitor cells into pit secretory cells. However, whether it plays a role in regulating mucosal antimicrobial defense is still unknown and we now investigate how the downregulation of BMP signaling affects mucosal inflammatory responses.

Exploiting 3D organoids, we demonstrate that epithelial cells activate NK- κ B signaling in response to microbe-derived proteins, while this effect is inhibited upon BMP signaling activation. In vivo, inhibition of BMP signaling in the epithelium via knockout of *Bmpr1a* (*Bmpr1a*KO) not only causes gland hyperplasia but also strongly enhances inflammation at the pit of gastric glands, where it is absent in mice with functional BMP signaling. We explore the consequences of increased inflammation upon epithelial *Bmpr1a*KO, demonstrating via whole transcriptome analysis that this is linked to enhanced antimicrobial tissue responses and that KO mice show reduced *H.pylori* colonization. Moreover, we discover that the influx of IL-1 β producing cells observed upon BMP signaling downregulation has an impact on stromal cells, which in turn produce pro-regenerative factors causing epithelial reprogramming into a highly proliferative fetal-like regenerative state. We demonstrate that *Il1r1*KO mice, where stromal cells lack the ability to respond to IL-1 β , do not show fetal-like reprogramming and are more colonized by *H.pylori*.

In conclusion, chronic *H.pylori* infection causes tissue reprogramming into a fetal-like state via a crosstalk between epithelial, stromal and immune cells. This state is linked to an enhanced proinflammatory response, increased proliferation and boosts antimicrobial defense. We propose that long-term activation of this state may enhance the risk for malignant transformation.

6

In-Depth Characterization of Lung Organoids: An Imaging-Multi-Omics Approach

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Understanding the characteristics of human lung organoids is essential for their application in modeling the human lung and studying its (patho-)physiology and host-pathogen interactions. However, their increasing complexity and cellular diversity amplify challenges such as donor- and batch-to-batch variability. To overcome these issues, standardized quantification of organoid complexity and heterogeneity at the molecular, cellular, and morphological levels is needed.

We present an integrative approach to characterize adult stem cell (ASC)-derived human lung organoids generated from ten donors over thirteen passages and nine months of culture. Our workflow combines imaging, image segmentation, single-cell, and spatial transcriptomics. Using multiplex immunofluorescence, high-content scanning, and semi-automated segmentation, we assess the cellular composition via characteristic markers benchmarked against reference lung samples. Morphological features such as organoid size, number of cells, and lumens per organoid are quantified from histological sections and compared to 3D data from fixed and live light sheet microscopy.

To further dissect cellular and molecular composition, we perform single-cell RNA sequencing and spatial transcriptomics. Spatial maps allow for cell type-resolved comparisons based on a selected set of transcripts between lung and organoid samples, enabling deeper insight into spatially organized cell populations.

Our analysis pipelines support a standardized, multi-scale characterization of organoids at the protein, transcriptome, and structural levels. This strategy is broadly applicable across organoid systems and improves the understanding, reproducibility, and development of complex organoid models for biomedical research.

7

Brain organoid technologies to model human brain diseases

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Understanding how the human brain function in both health and disease remains one of the greatest challenges in modern science, yet hindered by limited availability of human samples and ethical restrictions.

In recent years, three-dimensional human brain organoids have emerged as a groundbreaking experimental system that overcomes many of these limitations. Derived from human pluripotent stem cells, brain organoids are genetically tractable and capable of recapitulating key aspects of early brain development, including cellular diversity, spatial organization, and functional properties reminiscent of the fetal brain.

We will provide an overview of recent developments in the brain organoid platform and showcase several exemplary projects that illustrate how this technology can be leveraged to model human brain diseases. These include applications in the study of neurodevelopmental disorders, neurodegenerative diseases, and brain infections, highlighting the potential of organoids to transform our understanding of brain pathology and support the development of novel therapeutic strategies.

8

Generation of an in vitro model to study infection with genotoxic bacteria on primary colonic epithelial cells

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Colorectal cancer is one of the most prevalent cancer types in Germany and despite various improvements in therapy, it is still associated with high morbidity and mortality. Understanding the early events and factors that drive this malignancy is essential for developing effective prevention strategies. Microbiota-driven genotoxins, such as Colibactin from *E. coli* have been directly linked to colorectal carcinogenesis. However, how colibactin affects different colonic cell types in homeostasis and regeneration is not well understood. Therefore, to address this gap in the field, we established an in vitro system to study colibactin-induced DNA damage and repair in a cell-type specific manner. In this model, colonic primary cells are cultured as organoids and transferred to transwell inserts under air-liquid-interface (ALI), allowing them to polarize. Under these conditions, the basal cell side is exposed to the medium and the apical side faces the air, resembling the intestinal lumen. This configuration is particularly suitable for studying host-pathogen interactions, as it maintains an accessible luminal surface for bacterial exposure. By taking advantage of lineage tracing (fluorescently labelled with tdTomato) and precisely titrating medium components and timing, we generated ALI cultures with three distinct cell populations: (1) proliferative stem cell/progenitors (Ki67+), (2) differentiated cells, composed mostly of colonocytes (Krt20/tdTomato+) and (3) regenerative state with de-differentiating enterocytes (Ki67+, Krt20/tdTomato+). Our preliminary results indicate striking alterations in DNA damage response to colibactin in different cell populations. The outcome of this research will significantly deepen our understand of DNA damage and repair in different regenerative cell states and could lead to novel approaches to prevent colibactin-associated cancer.

9

Full-thickness skin equivalents as a model to study skin-T cell crosstalk using spatial imaging

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CD8⁺ and CD4⁺ T cells exert highly specialized roles in adaptive immunity. Cytotoxic CD8⁺ T cells kill infected and malignant cells while CD4⁺ T cells express the helper molecule CD40L - critical for APC licensing and B cell class switching. We previously demonstrated that memory CD8⁺ T cells exhibit chemokine receptor profiles similar to those of CD4⁺ T cells, allowing their classification into corresponding Th/c1, Th/c2, Th/c17, Th/c17+1, and Th/c22 subsets. Notably, the Tc2, Tc17 and Tc22 CD8⁺ memory T cell subsets lack features of cytotoxicity and instead express CD40L upon activation. These CD8⁺ “helper” T cells display a skin migratory signature and are enriched in the blood of psoriasis patients, indicating a potential role in skin inflammation. To study the individual and combined contributions of CD4⁺ and CD8⁺ T cell subsets to psoriasis development, a physiologically relevant *in vitro* model that captures the complexity of human skin and its immune landscape is essential. For this purpose, we generate full-thickness skin equivalent (FTSE) models composed of primary fibroblasts and keratinocytes derived from adult or juvenile donors and incorporate allogeneic *in vitro* polarized CD4⁺ and CD8⁺ T cells. Next, we aim to advance into an autologous FTSE model to enable direct comparisons between healthy and diseased skin and facilitate the integration of additional immune cell populations. We characterize the tissue architecture and immune cell infiltration of our model using histology and high-plex spatial imaging with the MACSima Platform. Utilizing cyclic staining technology, we can monitor multiple marker expressions and psoriasis-associated phenotypic changes, such as epidermal differentiation and thickening within one tissue section. By dissecting the distinct contributions of individual T cell subsets to chronic skin inflammation, this approach may pave the way for more targeted and effective therapeutic strategies.

10

Modular open-source exposure platform: 3D-printed *Klemmbaustein*system for toxicity assessment of fungal metabolites in Air-Liquid-Interface (ALI) cultures

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Current exposition research for volatile organic compounds relies on expensive commercial devices that limit accessibility and customization. We developed a new open-source, 3D-printed Air-Liquid-Interface (ALI) exposure system that liberates respiratory toxicology research by providing a cost-effective, highly adaptable alternative to proprietary established devices. The modular system offers standardised dimensions based on modular blocks with flexible configuration blocks for 6.5 mm TransWell inserts. Key features include lateral gas channels to ensure even distribution, channels for integrated water circulation allowing precise temperature control at 37 °C, and optional perfusion channels for automated medium exchange or long-term exposure studies. The 3D-printed sealing lid secured with M4 screws ensures leak-free operation while maintaining the modularity of the system for parallel experiments.

Using immortalized human airway basal cells (BCI-NS1.1) cultured for 28 days in ALI conditions, we validated system performance through formaldehyde pulse exposure experiments. The system effectively delivered approx. 0.2 µg formaldehyde (~2.1 ppm), demonstrating that volatile substances pass through efficiently and significantly affect cultured cells. Ciliary beat frequency was used for sub-toxic validation and it reduced from 7.2 ± 1.8 Hz to 5.2 ± 1.2 Hz while maintaining 80-90% cell viability.

This open-source approach is changing exposure research by reducing dependence on proprietary manufacturers and enabling laboratory-specific customisation. The system's high adaptability and range of applications extends not only to fungal VOCs, but also to potentially hazardous and industrial gases. By providing free access to this standardised exposure technology, we facilitate the comparison of results between different laboratories and accelerate respiratory exposure research through shared system improvements and accessible, reproducible methods.

11

Multi-electrode array recordings of brain organoid network activity and plasticity

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The brain organoid field has significantly expanded in recent years, leading to a variety of organoid types and methods to generate them. New brain organoid models are often validated solely by transcriptomics to test if they have similar cellular composition as the brain area they are intended to represent. But cell-type composition based on gene expression does not necessarily predict function. For example, the presence of inhibitory neuronal markers does not necessarily mean that inhibitory neurons are present and function normally. It is therefore important to also test brain organoid function, in terms of network activity, firing properties, and responses to known pharmacological agents. Here we use high-density multi-electrode array (MEA) recordings to compare forebrain and hippocampal-like organoids derived from two different donor lines (HUMIMIC106 and TMOi001-A). We found differences in spontaneous activity between both organoid type and donor line. In addition, forebrain and hippocampal-like organoids responded differently to gabazine, which blocks inhibition by binding to GABAA receptors, with hippocampal-like organoids showing a larger response. This suggests that there are higher numbers of functional inhibitory neurons in hippocampal-like organoids. In preliminary tests in one donor line (HUMIMIC160) we induced chemical LTP with glycine. Both forebrain and hippocampal-like organoids showed potentiation for at least 90 minutes after induction, suggesting that they are also capable of long-term synaptic plasticity.

12

MicroRNAs shape early neural fate through a critical time window in human brain organoids

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MicroRNAs (miRNAs) are small non-coding RNAs with crucial roles in cell-type specific gene regulation and particularly abundant in the mammalian brain. While extensively studied in animal models, their role in regulating early neurodevelopment has never been systematically explored in a complex human context. To address this, we profiled mRNA and miRNA expression during human forebrain organoids (HFOs) development and maturation. Developing HFOs display miRNA signatures mirroring human neurogenesis progression: neuronal miRNAs such as miR-9, miR-124 and the let-7 family, were enriched at this stage, while stem cell-specific miRNA families, such as miR-302, were depleted. We found that miRNA expression closely correlates with human fetal datasets, as the HFOs matured. We observed oscillatory expression patterns of both miRNAs and key components of the biogenesis machinery (DROSHA, DICER, AGO2), which peaked during neural commitment and declined upon differentiation. To dissect miRNAs temporal requirement, we globally downregulated them at two developmental stages- neural induction (NI) and neural differentiation (ND)- by overexpressing a dominant-negative DROSHA mutant. NI perturbation led to widespread dysregulation of miRNA and transcripts related to morphogen signaling pathways, particularly WNTs. In contrast, the effects in ND-perturbed HFOs were markedly milder, suggesting a critical time window for miRNA-mediated regulation. Single-cell RNA-seq further evidenced a pronounced shift in developmental fate, diverging from forebrain identity more prominently in NI-perturbed organoids than in ND-perturbed ones. Overall, we provide the first comprehensive investigation of miRNA-mediated gene regulation in controlling early events of human brain development. Our data suggest an essential requirement of miRNAs for the timely regulation of neurogenic commitment.

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BIH Core Unit Pluripotent Stem Cells and Organoids (CUSCO): Advancing Human 3D Organoid Models

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The BIH Core Unit Pluripotent Stem Cells and Organoids (CUSCO) is a state-of-the-art facility dedicated to advancing human 3D organoid models for biomedical research. CUSCO supports basic and translational research by facilitating all aspects of human pluripotent stem cell (hPSC) and organoid technology. This includes the generation of human induced pluripotent stem cells (hiPSCs) from somatic samples, their genetic manipulation (e.g., CRISPR/Cas9 genome editing), thorough characterization and quality control of hiPSC lines, and distribution of validated lines to researchers. CUSCO also offers expert training, standardized protocols, and consultation to ensure proper handling and reproducible experimentation with hPSCs and organoids.

A major focus is the development and refinement of differentiation methods to drive hiPSCs into specific cell types and complex 3D organoids. Using these protocols, CUSCO has established organoid models of multiple human tissues (e.g., brain, liver, heart) for disease modeling and drug discovery. Cutting-edge analytic platforms, such as single-cell sequencing and high-content imaging, are integrated for in-depth characterization of organoid development and function.

The unit continuously innovates, with internal R&D projects aimed at improving reproducibility of organoid-based assays and establishing vascularized organoid platforms. Together, these services and developments position CUSCO as a central hub empowering researchers to utilize human 3D organ models, accelerating biomedical discoveries while adhering to the 3R principles of Replacement, Reduction, and Refinement in animal experimentation.

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Pathogen-host interactions and pharmacological testing using human lung tissue cultures and organoids

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Disease Background: The use of primary human lung tissue for ex vivo lung disease modeling offers valuable insights into lung infections and supports translational research. Given the host specificity of many human lung pathogens, understanding the molecular interactions between viruses, bacteria, and lung target cells is essential. In vitro lung organoids derived from adult human lung stem cells provide a scalable and long-term source of patient-specific material.

Models: Ex vivo human lung tissue explants are employed with common respiratory pathogens, such as influenza A virus (IAV), SARS-CoV-2, and *Streptococcus pneumoniae*. Additionally, alveolar- and bronchial-like organoids are used. These models are well suited for analyzing viral replication, performing drug efficacy testing, and applying techniques such as spectral microscopy and single-cell RNA sequencing (scRNA-seq).

Major outcomes of our research: Viral and bacterial infections in these models revealed pathogen-specific effects on epithelial integrity and host cell types. SARS-CoV-2 and *S. pneumoniae* infections lead to distinct disruptions in tight junction proteins such as occludin and ZO-1, underlining the impact on barrier function. Organoids recapitulate key epithelial features of the lung and show differential responses depending on their alveolar or bronchial identity.

Future objectives: Ex vivo lung tissue remains a valuable reference model for studying infections. In vitro lung organoids offer the advantage of long-term culture, but further characterization is needed to increase their physiological relevance. A key goal is to develop immunocompetent organoid models, for example by incorporating alveolar macrophages. These advanced models will enable the study of viral and bacterial infections in a more representative environment.

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Combination of MALAT1-Targeting ASO (Antisense oligonucleotides) and Pembrolizumab in RCC Spheroids and Perfused 3D Co-Culture Models

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Renal cell carcinoma (RCC) remains a challenging malignancy with limited long-term survival rates and resistance to current immunotherapies. To explore novel combination strategies, we are investigating the effect of Pembrolizumab and an antisense oligonucleotide (ASO) targeting the long non-coding RNA MALAT1 in a 3D co-culture model of RCC.

Using 786-O RCC spheroids, a multi-condition setup including untreated controls, PBMC-only, ASO-only, Pembrolizumab + PBMCs, ASO + PBMCs, and ASO + Pembrolizumab + PBMCs is being established. Treatments were applied for 7 days, and tumor cell viability was assessed using IncuCyte NIR fluorescence imaging. MALAT1, which has been associated with tumor cell proliferation and metastasis, is upregulated in human RCC tissue. Silencing MALAT1 decreases RCC cell proliferation and invasion while increasing apoptosis. Therefore, MALAT1 is considered a potential therapeutic target to enhance treatment susceptibility in this malignancy.

In parallel, a perfused 3D co-culture model was established using the HUMIMIC Chip system to better mimic immune-tumor interactions under physiological flow conditions. This setup enables direct comparison between static spheroids and perfused tissue models in terms of immune responsiveness and treatment efficacy.

The project aims to evaluate the feasibility and potential benefit of MALAT1 knockdown in enhancing immunotherapeutic responses and to assess whether perfused models produce more physiologically relevant immune effects.

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Modeling the Blood-Brain Barrier Using Blood Vessel Organoids for Fluorinated Multiple Sclerosis Drug Investigation

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To investigate the penetration of fluorinated drugs through the blood-brain barrier (BBB), we are developing a physiologically relevant in vitro model of the human BBB. This is achieved by combining brain organoids with blood vessel organoids (BVOs) that recapitulate key structural and functional features of the BBB, including specialized endothelial cells, pericyte interactions, and basement membrane components.

The BVOs are derived from human pluripotent stem cells (hPSCs) and guided through differentiation protocols to form blood vessel networks within a 3D extracellular matrix environment (Wimmer et al., 2019). These organoids are then dissociated and overlaid onto cortical brain organoids, leading to an inside-out BBB like organoid, which mimics the cellular complexity of the in vivo BBB, making them a platform for drug penetration studies.

We are specifically interested in multiple sclerosis (MS) treatment strategies and would like to evaluate the pharmacodynamics of fluorinated compounds, such as Bruton's Tyrosine Kinase inhibitors (BTKi), which require efficient CNS delivery to have an effect on MS disease progression. The organoid BBB model enables analysis of fluorinated BTKi uptake and barrier integrity in response to drug exposure.

This approach offers a scalable, ethically responsible alternative to animal models and provides a high translational value for early-stage drug screening and BBB permeability studies, as well as having the opportunity to later on use patient-derived iPSCs to be able to test those drugs on a multiple sclerosis BBB organoid model. By combining this organoid model with magnetic resonance imaging, we aim to uncover the most effective fluorinated BTKi, that could then be used with the same imaging method on an animal model, allowing a fully transferable technique, while limiting the use of those animal models.

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Interleukin-1 β Induced Mucus Hyperconcentration Impairs Mucociliary Transport in Human Airway Cultures

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Introduction: Defective mucociliary clearance (MCC) is a hallmark of muco-obstructive lung diseases, characterized by mucus plugging, persistent inflammation, and recurrent infections. Effective MCC relies on ciliary motion, airway surface liquid, and a mucus layer. However, the link between inflammation and impaired MCC remains unclear.

Objective: We aim to investigate the impact of Interleukin-1 β (IL-1 β) on MCC in primary human nasal epithelial (HNE) cultures, a mediator of neutrophilic inflammation in diseases like cystic fibrosis and bronchiectasis.

Methods: Differentiated primary HNE cultures from healthy donors were treated with IL-1 β for five days. We assessed CFTR and SLC26A9 expression and transepithelial ion transport using Ussing chambers. Mucin (MUC5B and MUC5AC) expression and secretion were quantified by agarose Western blotting. Further, we measured mucus solids, ciliary beat frequency (CBF), and mucociliary transport (MCT) velocity.

Results: IL-1 β significantly increased CFTR (1.4-fold, $p < 0.05$) and SLC26A9 (1.6-fold, $p < 0.01$) mRNA expression, as well as chloride secretion (CFTR: 1.6-fold, $p < 0.05$; SLC26A9: 1.5-fold, $p < 0.05$). MUC5B transcript (13.3-fold, $p < 0.001$) and protein (5.1-fold, $p < 0.001$) were increased, with mucus solids rising to 12.3% (vs. 3.8%, $p < 0.05$). CBF (4.1 vs 5.0 Hz, $p < 0.001$) and MCT velocity (1.4 vs 6.6 $\mu\text{m/s}$, $p < 0.001$) were decreased with IL-1 β .

Conclusion: Our results show that IL-1 β enhances chloride secretion and mucus secretion. Elevated mucus solids and impaired MCT indicate that upregulation of chloride is insufficient to maintain mucus hydration in neutrophilic inflammation. As this may contribute to mucus stasis in chronic airway diseases, ongoing studies aim to elucidate the interplay between inflammation and MCC.

Mechanical strain exacerbates *Pseudomonas* infection in an organoid-based pneumonia-on-a-chip model

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To investigate early defense mechanisms against pulmonary pathogens, we utilize human in vitro models that closely mimic in vivo phenotypes, minimizing species-specific differences and adhering to the 3R principles.

Specifically, we employ a human alveolus-on-a-chip model (Emulate system) that replicates the epithelial-endothelial barrier while integrating key physiological factors, including air exposure, perfusion, and mechanical forces. Initially, we used commercially available primary epithelial cells but found they lacked essential alveolar characteristics, such as surfactant production. To overcome this, we incorporated adult stem cell-derived organoid cells, which, when cultured on the stretchable chip, exhibit features of mature surfactant-producing alveolar type 2 (AT2) cells and even increased expression of typical alveolar type 1-like (AT1) markers.

To explore infection dynamics between different stretch conditions and cell types, we established *Pseudomonas aeruginosa* infection on the chip, investigating how mechanical strain influences pathogen susceptibility and lung barrier damage. While hyper-physiological stretch (10%) alone did not compromise barrier integrity, infection with *P. aeruginosa* under 10% stretch increased permeability and vascular bacterial burden compared to infection under physiological stretch (5%). This suggests that enhanced mechanical strain exacerbates *Pseudomonas* infection.

In summary, our *Pseudomonas* pneumonia-on-a-chip model demonstrates that infection progression varies with epithelial cell type and mechanical strain. Moving forward, we aim to further dissect stretch-dependent mechanisms of infection-induced barrier disruption and integrate host immune effector cells into the model.

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Electrophysiological evaluation of salivary gland barrier and transport properties

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Salivary secretion is a highly regulated process that integrates receptor-mediated signaling (muscarinic and adrenergic) with downstream Ca^{2+} and cAMP pathways to orchestrate ion and fluid transport. Mutations in *Claudin-10* (*Cldn10*), a tight junction protein, are implicated in rare human disorders such as HELIX syndrome, characterized by hypohidrosis, electrolyte imbalance, and salivary gland dysfunction. Our hypothesis is that Claudin-10b deficiency causes a reduction in Na^+ secretion in the acini of the salivary gland, resulting in a reduced saliva volume.

To investigate this, we established a robust organoid model from wild-type mouse salivary glands. Organoids were cultivated both in 3D Matrigel and in 2D culture on cell culture filter inserts, enabling functional assessment in Ussing chamber experiments. Stimulation with carbachol, isoproterenol, and forskolin elicited measurable electrophysiological responses, while specific inhibitors — such as Diphenylamine-2-carboxic acid, flufenamic acid, and bumetanide — provided insights into the underlying ion transport mechanisms.

Our current data demonstrate the functional relevance of these models for dissecting the salivary secretion pathway and establish a baseline for future studies using *Cldn10*-mutant organoids. These findings give the way for understanding how tight junction defects contribute to altered glandular function.

Micro Cavities for the Scalable Production of Filament Induced Brain Organoids

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Generating 3D brain organoids is challenging. The complex spatiotemporal mimicking of the brains development is achieved by unguided protocols administering only an impulse via brief chemical intervention and the provision of scaffolds/filaments to drive cells into the neuroectodermal lineage while subsequent differentiation occurs spontaneously. This leads to a high inter-organoid/batch variability. Guided protocols with strong chemical intervention are more reproducible, but often lack the required spatiotemporal complexity. Here, we introduce the novel scaffold system MatriFilamentSystem® (MFS) that combines both, reproducibility and the spatiotemporal complexity. More than 1000 MFS embryoid bodies (EBs)/organoids per batch are produced as compared to less than 100 in conventional protocols. The MFS is easy to handle, but permits the combination of an intrinsic and extrinsic scaffold to combine reproducibility, scalability, and optimal differentiation. MFS organoids show comparable expression of SOX2, Nestin, PAX6, and MAP2 to those produced with unguided approaches. Likewise, morphology and spatial distribution of differentiating neuroepithelium is preserved in MFS organoids. However, MFS organoids can be reliably produced with as little as 600 cells/EB, about 15 times less than used in conventional approaches. In summary, the MFS combines micro/nanotechnology approaches with modern stem cell biology to attain improved, reproducible differentiation and easy handling.

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Human Developmental Liver Disease Identifies Innate Cytokine Control of Epithelial Identity and Regeneration

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Background: Inflammatory cues influence epithelial function during injury; however, their role in restoring and maintaining epithelial identity in development is less well understood. This is relevant in diseases like biliary atresia (BA), a severe neonatal disease marked by biliary epithelial injury and obliteration. Although a complex inflammatory phenotype in BA livers is well described, its impact on epithelial fate and developmental restoration remains elusive. Our study investigated the interaction between innate cytokine gradients and epithelial transcriptional programs in patients and organoids, utilizing a combination of clinical omics data and patient-derived organoid models.

Methods: Single-nucleus RNA sequencing (snRNA-seq) from clinically stratified BA and control liver samples (n = 12, n = 4 per group) identified cell states and transcriptional signatures in myeloid and biliary cells (cholangiocytes). Patient-derived intrahepatic cholangiocyte organoids (ICOs) from BA and control livers, thereby modeling pre-injured and baseline epithelial states, were treated ex vivo with cytokine gradients of TNF α and IL-1 β (ranging from 10 ng/mL to 100 ng/mL). Organoid morphology and growth patterns were assessed, and the epithelial transcriptional response was analyzed by gene expression using quantitative PCR (qPCR).

Results: Livers from BA patients with severe versus favorable clinical outcomes demonstrate that *IL-1 β* and *TNF- α* surge in the myeloid immune compartment in severe clinical cases (resulting in liver transplant). Additionally, cholangiocytes in severe disease exhibit an immune-reactive *SOX9*-high progenitor state. In favorable clinical outcomes, cholangiocytes activate an epithelial-repair state, characterized by transcription regulators such as *DACH1* and *BMPR1B*. In functional experimentation, ICOs from patients with BA activate a *DACH1*- and *BMPR1B*-high repair program only when exposed to low-milieu concentrations of IL-1 β and TNF- α , while high concentrations abolish this effect. Furthermore, these low levels of cytokines promote ICO expansion more than high cytokine or no cytokine-addition, indicating a tightly regulated response.

Conclusions: Innate immune signaling serves as a functional “inflammatory rheostat,” guiding biliary epithelial repair and injury.

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HUMAN NEUROMUSCULAR ORGANOIDS MODEL SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality. Motor neurons (MNs) are considered the primary target in SMA but retrograde signals from skeletal muscles and neuromuscular junctions (NMJs) can also be crucial players of the MN degeneration. Muscle atrophy is a hallmark of SMA, and a growing body of evidence suggests that muscle pathology could have an important role in this disease. However, the series of events that lead to MN degeneration in humans remains poorly understood. Here, we used induced pluripotent stem cells (iPSCs) from three different patients diagnosed with SMA type 1 to generate 3D neuromuscular organoids. SMA-NMOs were efficiently generated and self-organized into the neural and muscle compartments. However, we observed a loss in the number and functionality of neuromuscular junctions, largely recapitulating the phenotype observed in patients. To gain a deeper understanding of the disease phenotype, we analyzed the SMA-NMOs using single-nuclear RNA sequencing. This approach allowed us to identify genes regulating various neuronal and muscular functions that are perturbed in our disease model. Additionally, we tested two SMN2 splicing modifiers in our model and demonstrated their differential effects on both neurons and muscles using molecular and functional assays. Our findings demonstrate the potential of patient-specific NMOs for drug screening approaches, paving the way for developing treatments that target the early stages of the disease and thus improving the lives of patients.

Optimizing human-derived Vital Tissue Slices as a platform for personalized treatment of patients with head and neck cancer

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Despite recent advancements in anticancer therapies, patient responses to treatment remain variable. This emphasizes the need for reliable, personalized biological models to study therapy resistance. Patient derived ex vivo vital tissue slices (VTS) offer a promising platform that preserves individual tissue characteristics for anticancer therapy research. In this study, we developed a rapid, reliable method to generate and culture human head and neck cancer VTS from patient tissues, enabling downstream applications such as assessment of radiation response and characterization of immune cell composition.

We successfully generated 9 VTS models from 10 tissue samples, mostly from patients with squamous cell carcinoma of oral cavity (take rate: 90%). Three culture media compositions were tested for their impact on cell viability: (i) advanced DMEM/F12 with FCS, Penicillin/Streptavidin, and HEPES; (ii) the same composition with bFGF and EGF; and (iii) further supplemented with IL-2, -7, -15. Three mechanical and enzymatic tissue dissociation protocols were tested on two uncultivated samples; CD45+ cell viability was assessed.

The interleukin-enriched medium showed improved viability of immune cells for up to 72h (n=9, p=0.04). For irradiation experiments, VTS were cultured for 24h before exposure to 0, 4, or 8 Gy. DNA damage response via γ H2AX foci formation assay was assessed by immunofluorescence. Flow cytometry analyzed immune cell composition and viability. In unirradiated controls, CD45+ cell viability ranged from 70–90% (n=2), dropping to 60–90% at 4 Gy (p=0.01) and 55–90% at 8 Gy. The viability of CD45+ cells remained above 75% in two of the dissociation protocols with no statistically significant differences observed between the three protocols (p=0.31; p=0.41; p=0.35).

This feasibility study supports the use of VTS to explore radiation effects on immune cells in 3D human tumor tissue models. As a 3R-aligned approach, VTS platform enables functional and mechanistic research into therapy resistance, advancing animal-free, precision oncology platforms.

Development and Drug Screening of a 3D bioprinted Co-Culture Organoid Model for Head and Neck Squamous Cell Carcinoma

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Patient-derived organoids (PDO) have become valuable preclinical models, however their lack of tumor microenvironment (TME) components, such as immune cells and cancer-associated-fibroblasts (CAF), limit their ability to recapitulate *in vivo* tumor behavior and predict therapeutic response. Current co-culture models fall short in reproducing the spatial characteristics of tumors, since the original architecture and cell interactions are lost, and some *in vivo* scenarios are difficult to achieve (i.e. tumor immune excluded tumors).

The aim of the project is to first understand how the bioprinter technology affects cells viability (tumor, fibroblasts and immune cells), and how the spatial organization of the different cells affects their phenotype and cell-cell and cell-matrix interactions.

To address these limitations, we are developing a 3D bioprinted model with several primary tumor cells and cell lines (i.e. head and neck and breast cancers), fibroblasts and immune cells. The tumor cells and fibroblasts are being bioprinted in several matrices, and compared with the traditional seeding protocols to understand the effects on cell viability (CellTiter-Glo® and live/dead assay) and phenotype (multiplex immunofluorescence). After this characterization, the tumor cells will be bioprinted and will include structurally organized TME components to further characterize the cell-cell and cell-matrix interactions with the same aforementioned methodologies. Drug response to targeted and immune therapies are evaluated and compared to traditional PDO models.

Across two cell viability assays, the bioprinted group showed comparable performance to the manually seeded group: Organoid size did not differ significantly between the bioprinted and manually seeded groups ($8781 \pm 1086 \mu\text{m}^2$ vs. $8665 \pm 1177 \mu\text{m}^2$; $p = 0.846$). Live/dead staining showed comparable live cell signals based on Calcein AM intensity ($2.22 \times 10^8 \pm 2.63 \times 10^7$ vs. $2.27 \times 10^8 \pm 1.42 \times 10^7$; $p = 0.672$), and CellTiter-Glo assays showed similar metabolic activity ($1.55 \times 10^6 \pm 9.4 \times 10^4$ vs. $1.60 \times 10^6 \pm 6.8 \times 10^4$; $p = 0.266$). Defined structures have been printed successfully using Cellink Start bioink. Optimization of culture conditions for each cell type is undergoing, and a immunofluorescence pipeline is under development.

We aim to establish a spatial organized bioprinted model that mimics the TME more accurately via 3D biofabrication, and perform comparative drug screening to assess whether spatial construction of TME enhances therapeutic prediction accuracy.

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Colon assembloids recapitulating self-organization of epithelial crypts and their resident stromal and immune niche

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In the gastrointestinal tract, the stromal and immune compartments have been increasingly recognized for their critical roles in supporting epithelial homeostasis. Concurrently, the organization of the mucosal microenvironment is also regulated by signals derived from the neighboring epithelium. Recently, we have established a novel gastrointestinal 3D co-culture system known as colon assembloids to study interplays between the epithelium and its niche. The assembloid combines murine colon organoids with complex stromal subpopulations in a unified structure. This system faithfully recapitulates the anatomical morphology and cellular composition of colon epithelial crypts and also the compartmentalization of their stromal niche. Our data indeed provide evidence for constant crosstalk between the epithelium and stroma along the crypt axis, which impacts not only the differentiation of the epithelium but also the positional identity and function of the neighboring stromal cells. Moreover, we have managed to enrich and maintain colon resident immune cells in assembloids, and we now investigate their identity and function. Taken together, the sophisticated self-organization of the epithelium and its niche in assembloids highlights the crucial role of their crosstalk. This tractable multiple-cell subtype assembly technique facilitates in vitro investigations into direct interplays among the colonic epithelium, stroma, and resident immune system.

Establishing a Patient-Derived Organoid Platform for High-Throughput Drug Screening and Radiosensitizer Discovery in Head and Neck Cancer

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Head and neck squamous cell carcinoma (HNSCC) remains a clinically challenging malignancy, characterized by high recurrence rates and limited systemic treatment options. Although radiotherapy is a cornerstone of curative treatment, its effectiveness could be improved through the identification of radiosensitizers and predictive biomarkers of radiation response. However, traditional preclinical models often lack biological relevance or scalability, thereby limiting translational progress.

We are developing a high-throughput drug screening platform based on patient-derived organoids (PDOs) to facilitate radiosensitizer discovery and inform personalized combination therapies. In this system, tumor cells are seeded as 100-cell droplets in 1.5 μ L Matrigel onto Cellvivo® 384-pillar plates using the ASFA® Spotter—a non-contact dispenser that enables miniaturized 3D cultures under tightly controlled conditions.

Post-irradiation responses are quantified using a multi-assay approach, including Calcein AM staining for cell viability, ATP-based luminescence assays (CellTiter-Glo®), and γ H2AX immunofluorescence imaging to assess DNA double-strand breaks. High-content imaging is conducted using the ASFA® Scanner, and data are analyzed via AI-powered pipelines to ensure objective and reproducible interpretation.

To account for inter-line heterogeneity among PDOs, assay timing is normalized to each line's specific doubling time, ensuring biologically meaningful comparisons of intrinsic radiation sensitivity.

The platform is currently undergoing technical validation and workflow optimization to ensure reproducibility and scalability. Upon successful establishment, it will be applied to radioresistant PDO models with matched clinical treatment histories. We intend to screen clinically approved compounds to identify alternative combinations that may surpass standard platinum-based radiochemotherapy in selected patient subgroups.

This PDO-based platform represents a high-throughput, ethically responsible, and clinically relevant tool for investigating drug–radiation interactions in HNSCC. Furthermore, it may serve as a translational blueprint for precision radiotherapy development in other solid tumors.

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Establishing a human organoid-based model of liver transplant rejection with regulatory T cells as an immunomodulatory strategy

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Despite progress in liver regeneration research, liver transplantation remains the only curative option for end-stage liver diseases. To prevent T cell-driven acute allograft rejection, patients require long-term immunosuppressive therapy, such as calcineurin inhibitors. However, these drugs are associated with serious side effects, including organ toxicity, elevated risk of severe infection as well as secondary malignancy. This underscores the need for innovative strategies to study rejection mechanisms and test alternative therapies. Regulatory T cell (Treg)-based therapy is a promising approach that harnesses the body's natural immunomodulatory mechanisms to suppress graft rejection. Clinical trials have shown encouraging outcomes, but further development depends on robust, human-relevant preclinical models.

Organoid technology offers a valuable platform for modeling liver diseases, enabling the study of physiological and pathological processes in a controlled environment. In our study, we aim to establish a 3D liver acute allograft rejection model that includes the most relevant target cells of the disease: cholangiocytes and endothelial cells. These cells are among the first to be attacked by allogeneic T cells, leading to hallmark features of rejection such as endothelialitis and ductular injury.

Our project aims to develop a physiologically relevant model by incorporating patient-derived autologous intrahepatic cholangiocytes and endothelial cells into 3D aggregates, and subsequently co-culturing these assembloids with HLA-mismatched T cells to induce an immune response.

Thus, we aim to provide a novel organoid-based liver allograft rejection model as a testing platform for new therapeutic approaches, such as Treg-based therapies, while reducing the reliance on *in vivo* models.

The impact of carbohydrate complexity on host-microbe interaction

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This project investigates how glycosylation of mucus in the gastrointestinal (GI) tract influences bacterial infection and gut health. The protective mucus layer, primarily composed of mucins, plays a critical role in maintaining the integrity of the epithelial barrier, supporting nutrient absorption, and defending against pathogens. Disruptions in this layer—such as those caused by pathogenic bacteria like *Helicobacter pylori* in the stomach or enteropathogenic *E. coli* in the intestine—can lead to diseases including gastritis and intestinal damage. In contrast, beneficial bacteria like *Lactobacillus reuteri* help strengthen the mucosal barrier.

To better understand these interactions, the project analyzes the glycosylation patterns of mucus produced by human organoid cultures, assessing how closely they replicate the native GI environment. By comparing different culture systems—such as monolayers and air-liquid interface models—the study aims to identify which best mimics *in vivo* conditions. Furthermore, genetically modified organoids lacking specific mucins (MUC2 and MUC5AC) or inhibitors that stop glycosylation will be used to determine the role of mucus in bacterial colonization and host response.

The two main goals are: (1) to establish infection models for key stomach and intestinal bacteria, and (2) to explore how individual glycosyltransferases and mucins affect these host-pathogen interactions. Ultimately, this research will shed light on the importance of mucus glycosylation in preventing or permitting infection, contributing to a deeper understanding of GI diseases and potential therapeutic strategies.

How to build better vasculature to help stroke patients

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Arteries are highways that deliver blood rich in nutrients and oxygen to the brain. When such a highway is blocked during a stroke, the blood supply is interrupted. This can be life-threatening. But, the blood can detour through collateral blood vessels and reach the affected brain areas. Having more collaterals is better for the patients, but the number of collaterals varies between individuals. How collaterals form and function is of great interest to academic and clinical researchers. Answering what can be done to make more collaterals in stroke patients translates into the discovery of stroke prophylaxis. Rab GTPase-effector binding protein 2 (Rabep2) is currently the most promising target for developing such prophylaxis. That is because it is responsible for the majority of variability in collateral extent. Yet, how to use Rabep2 as a therapeutic remains unknown. That is because we do not understand how Rabep2 works and why its function is needed for collaterals. Moreover, we lack a robust research model to answer such questions. I aim to bridge these knowledge gaps and resolve the missing principles underlying the specificity of Rabep2 to collateral formation. To do that, I will use endothelial cells cultured as 2D cellular monolayers and as 3D vasculature-on-chip and a spectrum of live-cell microscopy, genetic engineering, and molecular approaches. I will also establish a platform for long term vasculature-on-chip culturing by developing a controllable and continuous flow system to overcome a major bottleneck in the field. Lastly, I will develop a collateral-on-chip model. It will allow resolving Rabep2 function in collaterals and provide researchers and clinicians with a robust method to test how to induce or improve collateral formation and make them adapt better after stroke. This research is necessary to guide a realistic hypothesis focused on testing Rabep2's therapeutic potential in animal models and patients.

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Studying neocortical development in human cerebral organoids

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Neural progenitors are the biological source of cellular diversification in the neocortex. Evolutionary expansion and morphological shaping of the human neocortex has been driven by a prolongation of the proliferative period of these cells and an increase in their diversity.

Here we investigate the mechanisms that regulate maintenance of the highly proliferative early neural progenitor subtypes and regulate generation of subsequent progenitors of limited proliferative capacity during development of the mammalian neocortex. We use in organoid electroporation (IOE) in human cerebral organoids to compare events during human and murine development and identify DTX4, a Deltex family member, as an evolutionarily conserved molecular determinant of neural progenitor identity. Preservation of radial glia identity, a highly proliferative progenitor subtype, is dependent on DTX4 expression. On the other hand, loss of DTX4 is a prerequisite for the generation of the poorly proliferative intermediate progenitors. Perturbations in DTX4 expression in human cerebral organoids and in the murine neocortex, alter progenitor composition, thereby inducing changes in neuronal diversity and cortical morphology. Our findings further highlight the critical role of cell cycle dynamics and of DTX4 in determining progenitor identity and thereby defining neuronal outcome during mammalian development.

Enhancing the Clinical Relevance of Preclinical Research: From Gold Standards to New Methods

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Complex 3D organ models such as organoids and organ-on-chip systems are increasingly recognized as tools to complement and, in selected cases, replace animal experimentation. However, for these models to be widely accepted by researchers and regulatory authorities, they must not only match but exceed the performance of "gold standard" animal models in terms of robustness, reliability, and clinical relevance. This poses scientific and practical challenges.

Our project addresses these challenges by building on the 6R framework developed in the context of animal research. The 6Rs expand the traditional 3Rs (Replace, Reduce, Refine) to include Robustness, Registration, and Reporting. We adapt and apply these principles to the development and evaluation of *in vitro* models, aiming to avoid known pitfalls of preclinical animal research such as overstated findings due to methodological flaws, poor reproducibility, and lack of transparency.

As part of this broader effort, we aim to harmonize key outcome measures across preclinical models of selected diseases, focusing on both animal and organoid model systems. By aligning these outcomes with core outcomes from clinical studies, we aim to increase the translational relevance of preclinical findings, and to identify areas where organoid and animal models can provide complementary insights. Through this integrative approach, we aim to foster trust in both gold-standard and novel approaches, and ultimately contribute to more responsible and clinically meaningful biomedical research.

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Characterization of Circulating MicroRNA Signatures During the Transition to Castration Resistance in Prostate Cancer

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The transition to castration-resistant prostate cancer (CRPC) is characterized by alterations in microRNA (miRNA) secretion. This study investigated whether *in vitro* culture conditions mimicking androgen deprivation could induce a CRPC-like miRNA profile in LNCaP spheroids. LNCaP spheroids were cultured for 30 days using an organ-on-a-chip system with agar-collagen gels under four conditions: 10% fetal calf serum (FCS), 10% stripped FCS (androgen-deprived), 10% human platelet lysate (HPL), and 10 nM dihydrotestosterone (DHT). Preliminary results indicate that androgen deprivation does not significantly reduce cell viability over 30 days. While PSA levels showed a slight upward trend across all conditions, stripped FCS exhibited a transient peak at day 15 with an amount of 59.9ng/ml, suggesting potential early changes in PSA expression under androgen deprivation. MicroRNA sequencing is currently underway to determine whether these conditions induce alterations in miRNA secretion profiles. These findings will guide future efforts to identify specific miRNA alterations involved in CRPC progression and explore their potential as biomarker.

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

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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 model *in vitro*, we established and characterized a human *in vitro* bone model, subsequently using methylprednisolone to induce GIOP and later treating it with anti-osteoporotic drugs.

As a basis, mesenchymal stromal cells (MSCs) were seeded onto a β -tricalciumphosphate (β -TCP) scaffold and differentiated into osteoblasts. Afterwards, human osteoclasts, differentiated from CD14⁺ monocytes, were added, and for induction of osteoporosis, treated with 10⁻⁶ M methylprednisolone (MP). 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 stainings.

Analysis of the supernatant shows lower levels of osteoprotegerine (OPG) and phosphate whereas levels of calcium are higher when treated with MP alone in comparison with the untreated and subsequently antiosteoporotic treated models. μ -CT shows a decrease in the trabecular thickness and volume in the osteoporotic group, whilst increasing in surface. Even the known faster onset of action of denosumab compared to alendronate was confirmed by our results. The colonization of the scaffolds was successfully confirmed by immunofluorescence staining. All current results show us the functionality of the model.

To implement more clinically relevant markers of bone metabolism, we were planning to use tests for procollagen type 1 N-terminal propeptide (P1NP) and procollagen-I propeptid (PICP) as markers for bone formation. Tests for beta-crosslaps are planned for measuring bone degradation.

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 using methylprednisolone. As a proof of concept, GIOP was treated with established antiresorptive drugs showing an increase of osteogenesis markers.

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Using *ex vivo* human brain tissue as a species-specific brain model to study neuronal function: a multimodal approach.

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Incomplete DNA homology between species contributes to discrepancies when translating experimental findings from murine models to human clinical trials. Therefore, the use of human *ex vivo* tissue is essential for the direct investigation of human neurophysiology. Using human tissue for both basic and translational research enhances the reliability of experimental outcomes, enabling more accurate insights into human brain function and pathophysiology for clinical application. Our laboratory has been utilizing *ex vivo* human brain tissue as a research model since 2017, yielding unique and valuable results. This approach not only provides a rare opportunity to study human neurophysiological properties in a species-specific context but also significantly reduces the reliance on animal models. Handling viable human tissue presents substantial challenges, requiring continuous optimization of collection and preparation protocols. Implementing advanced techniques in this delicate model further expands the scope of experimental investigations. Among these, the innovative patch-seq technique stands out by enabling simultaneous analysis of cell morphology, electrophysiological properties, spatial localization, genetic background, and transcriptomic profiles. Applying patch-seq to *ex vivo* human brain tissue allows researchers to correlate multiple aspects of neuronal identity, establish functional-genetic links, and classify cell types based on comprehensive neuronal characteristics while avoiding species-specific genetic discrepancies from animal models.

CSF1R-dependent myeloid cells direct hepatocyte development in human and mouse models

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The embryonic liver harbors diverse progenitor populations and serves as a niche—for example, for bipotent hepatoblasts that differentiate into hepatocytes or biliary cells. Kupffer cells, the most abundant myeloid population in the liver, co-develop from yolk sac–derived hematopoietic progenitors. While myeloid-to-hepatobiliary crosstalk is well-studied in pathology, its role in hepatic versus biliary fate decisions during embryonic development remains elusive.

We utilized our lab's multi-tissue fetal liver organoids (FLOs), derived from human induced pluripotent stem cells (hiPSCs), which establish an autonomous niche supporting the differentiation of HepPar1+ AFP+ hepatocyte-like cells, CK19+ biliary-like cells, and IBA-1+ myeloid-like cells with yolk sac ontogeny.

To investigate the role of myeloid cells in liver development, we employed a multi-model approach to perturb myeloid development by:

- a) depleting CD45+ CD14+ myeloid cells in FLOs using both chemical CSF1R inhibition and CRISPR-mediated *CSF1R* knockout, and
- b) depleting yolk sac–derived macrophages in mice from embryonic day (E) 12.5 and E14.5 onwards using conditional *Csf1r* and *Spi1* knockouts, respectively.

In FLOs, depletion of CD45+14+ cells led to a reduction in the ALB+ PanCK- hepatocyte-like population ($p = 0.0015$) without affecting proliferation, and downregulation of the hepatocyte transcription factor and myeloid activation regulator *CEBPB* ($p = 0.039$) and the functional hepatocyte marker *haptoglobin* (*HP*, $p = 0.0003$). In vivo, early macrophage depletion from E12.5 significantly reduced *Hnf4a* expression ($p = 0.0306$), whereas depletion from E14.5 had no effect ($p = 0.0904$), indicating that macrophages promote hepatocyte fate during a tightly regulated developmental window.

In summary, human organoid and mouse models support a role for CSF1R-dependent myeloid cells in promoting hepatocyte fate from a bipotent progenitor pool. Future work, including single-cell RNA sequencing and functional validation in FLOs, will further define mechanisms of myeloid–hepatic crosstalk.

The Patient-Specific Modeling of the Colorectal Cancer Tumor Microenvironment in a reproducible, quantifiable and long term functional model system via Autologous Co-Culture of PBMCs, tumor organoids Cancer associated fibroblasts

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Colorectal cancer (CRC) is the second leading cause of cancer-related mortality globally, with high grade forms with – apart from surgery - limited therapeutic efficacy in microsatellite stable (MSS) tumors, which constitute the majority of cases. Unlike microsatellite instability high (MSI-H) or mismatch repair-deficient (dMMR) CRC, which are responsive to immune checkpoint inhibitors (ICIs), MSS tumors are characterized by a profoundly immunosuppressive tumor microenvironment (TME) that remains poorly modeled and understood. To address this clinical gap, we present a novel autologous in vitro platform that integrates key components of the patient's immune and stromal landscape. Peripheral blood mononuclear cells (PBMCs), patient-derived organoids (PDOs) from tumor and adjacent non tumor tissues, and cancer-associated fibroblasts (CAFs) are simultaneously isolated from individual CRC patients and co-cultured to recreate the native TME. This system enables the functional interrogation of immune activation, infiltration, and tumor directed cytotoxicity using real-time live-cell imaging (Incucyte), immune phenotyping (flow cytometry), and gene expression profiling (RT-PCR). CAFs are characterized for extracellular matrix (ECM) components, with a focus on collagen type X, a matrix molecule implicated in stromal-driven immunoregulation. Importantly, we incorporate assessment of alterations of extracellular pH—a metabolic parameter increasingly recognized as a regulator of cancer stem cell (CSC) phenotype and immune suppression. Acidosis in the TME, often driven by stromal remodeling and glycolytic CSCs, is hypothesized to impair T-cell function and skew macrophage polarization, thereby contributing to immune evasion. By capturing these patient-specific biochemical and cellular dynamics, this platform aims to overcome limitations of existing CRC models and support the development of precision immunotherapies, particularly for MSS CRCs currently resistant to ICI treatment. Our cell technology shall act to support companion diagnostics in future ONKO-ZERT co-clinical trials.

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A cell-permeable nanobody synergizes with CFTR modulators and restores F508del-CFTR function to near-normal levels in airway epithelial cells from patients with cystic fibrosis

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Cystic fibrosis is a life-limiting disorder affecting multiple organ systems. The majority of CF cases are caused by the *F508del* mutation within the CFTR protein. The current standard of care is a triple combination therapy consisting of small molecules elexacaftor/ tezacaftor/ ivacaftor (ETI), which restore CFTR function to ~50% of normal in patients with CF who carry at least one *F508del*-CFTR allele, improving clinical outcomes. However, persistent infection and inflammation highlight the need for further CFTR function improvement. We previously demonstrated that a nanobody (NB) stabilizes *F508del*-CFTR, but its impact on the functional restoration of ion transport remains unexplored due to challenges in efficient drug delivery across epithelial barriers.

We aimed to:

1. Achieve efficient intracellular delivery of NB using cell-penetrating peptides (CPP);
2. Evaluate the effect of NB on the functional rescue of *F508del*-CFTR;
3. Assess the synergy between NB and ETI.

CFBE410- cells expressing *F508del*-CFTR were treated with the NB conjugated to CPP via a disulfide bond (NB-CPP). Dose-response studies were conducted using transepithelial short circuit current measurements. Primary airway epithelial cells from three patients with CF homozygous for *F508del* were used to assess NB-CPP-mediated rescue of CFTR function and its synergy with ETI.

Conjugation with CPP enabled intracellular delivery of NB in CFBE410- cells, enhancing

F508del-CFTR rescue. In airway epithelial cells from patients with CF, NB-CPP led to a 1.9-fold increase in *F508del*-CFTR function ($p < 0.001$). Co-treatment with NB-CPP and ETI enhanced *F508del*-CFTR rescue 1.6-fold compared to ETI alone ($p < 0.001$), reaching 89% of normal levels.

Efficient delivery of NB-CPP highlights the utility of cell-permeable nanobodies as next generation biopharmaceuticals for modulating protein function. Combined with ETI, NB-CPP restores *F508del*-CFTR function to near-normal levels in airway epithelial cells from patients with CF.

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Charité 3R Primary Tissue Pipeline: Clinical waste into scientific gold to support biomedical research

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Human model systems, such as organoids and engineered tissues, serve as proxies for specific aspects of organ function. Patient-derived tumor organoids can capture oncogenic signaling within a patient-specific background, which can reveal mechanisms of therapy resistance. Additionally, advances in omics-based transcriptome, proteome, and metabolome analysis technologies offer invaluable tools for uncovering disease mechanisms and optimizing therapies. When combined with clinical data, these methods can enhance the predictive value of preclinical research and expand the possibilities for personalized medicine, ultimately benefiting patients. A structured and comprehensive access to primary tissue is essential for these advancements.

As one of Europe's largest hospitals, Charité provides both patient care and performs extensive biomedical research. During surgeries and medical procedures, tissues and cells are excised and withdrawn from patients—though only partially utilized for diagnostics.

With the “Primary Tissue Pipeline”, we have established a central facility at the Charité – Universitätsmedizin Berlin that facilitates access to primary tissue for Charité researchers and their collaborators. It serves as an interface between Charité as a hospital and the biomedical research community. The pipeline supports specific research projects by initiating and coordinating the sampling of human tissue. Specifically, it contacts relevant clinical departments, recruits patients and suitable biosamples, supports with application for ethical votes and builds up a project-specific logistic for sampling. The initiative serves as a central point of contact and takes on a pilot function in order to make the best possible use of the existing infrastructures at Charité.

The Primary Tissue Pipeline is a service platform to provide (fresh and vital) primary samples and clinical data that might serve as a blueprint for similar approaches beyond Charité.

Mapping the interdependency of the gut-lung-axis and hydrogel barrier in health and disease

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The gut and lungs are anatomically distinct, yet potential anatomic communications and complex pathways involving their respective microbiota have unveiled the existence of a gut–lung axis (GLA). This inter-tissue crosstalk putatively contributes to healthy and diseased states. To explore these interactions, we employed a multi-organ-on-chip (OoC) system comprising human bronchial and intestinal epithelial microphysiological systems (MPS). To ensure suitability for analyzing human mucus dynamics, we first characterized a bronchial air-liquid interface (ALI) model. Epithelial differentiation and mucus production were assessed using immunofluorescence and hematoxylin and eosin (H&E) staining, along with time-resolved quantification of Muc5AC over three weeks. A heuristic design strategy was applied to systematically vary eight media components and identify a shared culture medium optimized for mucus production and compatible with both MPS. In the final setup, we induced an IBD-like phenotype in the gut model using TNF- α and IFN- γ , and assessed its impact on the connected lung model. Proteomic analysis revealed changes in lung mucus composition, including upregulation of pendrin, lactoferrin and dual oxidases—proteins associated with airway inflammation. These findings demonstrate that our OoC platform successfully recapitulates aspects of gut–lung axis communication and offers a valuable tool for investigating disease mechanisms and inter-organ signaling in vitro.

Paclitaxel induces cell-type composition changes and affects NCS-1 in induced pluripotent stem cell-derived brain organoids

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Neurotoxic phenomena are among the most common side effects of cytotoxic agents. The development of chemotherapy-induced polyneuropathy (CIPN) is a well-recognized adverse reaction in the peripheral nervous system, while changes of cognitive functions (post-chemotherapy cognitive impairment (PCCI)) are more diffuse and have only recently drawn scientific interest. PCCI in patients most often displays as short-term memory loss, reduced multitasking ability or deficits in language. Not least, due to a lack of preclinical human model systems, the underlying molecular mechanisms are poorly understood, and treatments are missing. We thus investigated whether induced pluripotent stem cell (iPSC)-derived brain organoids can serve as a human model system for the study of chemotherapy induced central nervous system toxicity. We robustly generated mature brain organoids from iPSC-derived neuronal precursor cells (NPC), which showed a typical composition with 1) dividing NPCs forming ventricle like structures 2) matured neurons and 3) supporting glial cells closer to the surface. When exposed to increasing concentrations of paclitaxel, a frequently used chemotherapy drug, we observed time dependent neurotoxicity with an EC₅₀ of 153 nM, comparable to a published murine model system. Western blot analysis after paclitaxel exposure demonstrated dose dependent degradation of neuronal calcium sensor one protein (NCS-1). We could also provide evidence that paclitaxel treatment negatively affects the pool of neuronal and astrocyte precursor cells as well as mature neurons. In summary our data suggests that human iPSC derived brain organoids are a promising preclinical model system to investigate molecular mechanisms underlying PCCI and to develop novel prevention and treatment strategies.

Keywords: 3R; brain organoids; chemotherapy; iPSC (induced pluripotent stem cell); neurotoxicity; new approach methodologies (NAM); paclitaxel.

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Modelling the intestinal mucus barrier in health and disease

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The intestine is site of various pathologies, ranging from infectious diseases to chronic inflammations, as well as cognate malfunctions. One key element affected in these malignancies is the intestinal mucus layer, located between the lumen and epithelium. The use of patient-derived intestinal organoids enables access to a model that captures both site- and patient-specific characteristics of the donors. While this model system has already been used to study various mechanisms of epithelial differentiation, a dedicated characterization of the *in vitro* generated mucus layer and the mechanisms involved in its generation is still lacking.

Here, we present a transwell filter-based model of the human colonic and rectal epithelial mucus layer that shares many characteristics with the *in vivo* barrier. Growth conditions that enable secretory cell generation in the colon produced viscous secretion, which was assessed both on a macro- and microscopical level. Rectal mucus generated from donors suffering from cystic fibrosis – a disease primarily affecting the lung – revealed that their secretion indeed was more viscous than healthy controls. In the colon, molecular analysis revealed that this secretion indeed contained acidic and neutral mucins, as well as various anti-microbial proteins and peptides, the expression of which can be modified by exposure to innate immune signals. Lectin staining revealed a glycosylation pattern low in fucose but high in *N*-acetylglucosamine and sialic acid. Further, our *in vitro* colonic mucus layer was found impenetrable to pathogenic *EPEC* bacteria.

We believe that these findings have strong implications on infectious disease research and beyond. The integration of our model in organ-on-a-chip systems to study complex networks such as the gut-lung-axis poses another application opportunity for our model. Our system thus promises to advance our understanding of disease mechanisms both within the gut and beyond.

Modeling CAR-T Cell Migration and Efficacy in a Microphysiological System

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Microphysiological systems (MPS) offer a promising platform to evaluate the efficacy and safety of T-cell-based immunotherapies, such as chimeric antigen receptor (CAR) T-cell therapies, *in vitro*. This study focuses on establishing an MPS to assess the motility and migration of CAR-T cells between endothelial and epithelial compartments, simulating tissue homing. Induced pluripotent stem cell (iPSC)-derived endothelial cells, donor PBMC-derived T cells as controls, and a transwell-based system were used to evaluate migration and cytotoxicity against CD19-expressing Nalm6 cells on-chip.

CAR-T cells were engineered via CRISPR-Cas9-mediated knock-in and expanded for functional testing. Migration capacities were investigated under varying chemokine gradients, and flow cytometry and imaging were performed to quantify migration and assess activation. Cytotoxicity was evaluated by measuring the relative abundance of CD19 wild-type GFP (WT) and knockout RFP (KO) Nalm6 cells in the apical compartment.

Key findings revealed that endothelial cells are essential for facilitating CAR-T cell migration, and chemokine gradients significantly influence motility. Notably, allogeneic CAR-T cells exhibited reduced migration compared to donor PBMC-derived T cell controls. Additionally, the presence of Nalm6 cells did not alter CAR-T migration properties but confirmed robust killing efficacy, as demonstrated by the selective depletion of CD19-expressing Nalm6 cells in the apical compartment.

This work highlights the potential of MPS to model immune cell migration and tissue-specific homing, specifically in the context of preclinical testing of CAR-T cell therapies. The platform provides a precision medicine approach by incorporating autologous systems and highlights the utility of MPS as testing platforms for next-generation cell-based immunotherapies, while reducing dependency of animal models.

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Renal Medullary Carcinoma – unexpected anti-tumor activity of immune cells in a rare tumor

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Renal Medullary Carcinoma (RMC) is a rare subtype of kidney cancer which accounts for less than 5% of all renal malignancies [1]. Nevertheless, it is the third most common renal malignancy among young adults and adolescents [2], being characterized by a poor prognosis and a low median overall survival (OS) of 13 months [3]. Aggressive tumors show rapid progression and dissemination, and no response to targeted therapies that are used for common subtypes of kidney cancer [2,4]. The lack of preclinical models enforces the difficulty of studying this rare tumor which underpins the relevance of establishing patient-derived organoid models (PDOs) mimicking the *in vivo* settings of this highly lethal tumor. The genetic composition of RMC tumors marks the patients as possible candidates for immunotherapy which rises an interest to study the native immune cell population of RMC patients [2]. To enable such experiments a modified protocol (mPDO) was developed in order to maintain the native tumor infiltrating immune cells (TIICs) and support their growth alongside the tumor cells. This would generate a more reliable model that resembles the 3D composition of tumors considering the substantial role of the tumor microenvironment (TME) in tumor formation, progression, invasion and treatment response [5], which was tested in several rare tumors. This work aims to establish long-term PDO cultures and characterize the TIIC population by flow cytometry to further analyze the dynamics that enabled the expansion of TIICs. Future experiments aim to gain insight into the behavior of the TME and their interaction with the tumor cells to ultimately predict response and resistance patterns upon drug therapy.

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Modeling intestinal absorption of apolipoprotein A-I Milano from genetically engineered rice using a 5D Intestine-on-Chip platform

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Organ-on-chip technologies are emerging as powerful alternatives to traditional in vivo models, offering human-relevant microenvironments, dynamic control of physiological parameters, and a significant reduction in the use of animals in experimentation. In the context of intestinal research, intestine-on-chip platforms allow for the study of digestion, absorption, and microbial interactions under highly controlled conditions that closely mimic human physiology. In this work, we used the IVTech LiveBox 5D dynamic system to study the intestinal transport and bioavailability of Apolipoprotein A-I Milano (AIM), an anti-inflammatory protein expressed in genetically modified rice. To replicate gastrointestinal conditions, the rice flour underwent simulated gastric digestion using the standardized INFOGEST protocol. The digested product was then incubated with *Bacteroides thetaiotaomicron* to simulate a simplified interaction with the microbiota. After this incubation, the supernatant was collected for quantification of AIM after bacterial exposure and evaluation of its cytocompatibility on Caco-2 monolayers. For transport studies, the supernatant was applied to the IVTech platform, which replicates key features of the intestinal barrier through its dynamic fluidic environment. Gene expression analysis of intestinal markers and barrier integrity testing using Yellow Lucifer dye confirmed tight junction formation and epithelial stability underflow. This intestine-on-chip model demonstrated enhanced physiological relevance and experimental reproducibility. This study highlights the potential of organ-on-chip platforms as a reliable approach for evaluating nutrient and peptide intestinal absorption, particularly in the development of functional foods and therapeutic delivery systems. Moreover, the intestine-on-chip approach consistently supports the application of "Reduction" and "Replacement" among the 3Rs principles.

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Analysis of airway commensals and their metabolites with respect to effects on CFTR modulator therapy

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The elxacaftor-tezacaftor-ivacaftor (ETI) triple combination CFTR modulator therapy has improved clinical outcomes for up to 90% of patients with cystic fibrosis (CF) who carry at least one F508del-CFTR allele. Evidence links a more diverse airway microbiome with improved clinical outcomes in patients with CF. The hypothesis suggests that some commensal bacteria may play protective roles in the airways by producing short-chain fatty acids (SCFAs) that inhibit pathogens and reduce inflammation.

To investigate the effects of SCFAs on F508del-CFTR function and their potential synergism with ETI, we performed transepithelial short-circuit current measurements using Ussing chambers in CFBE41o-cells. To study the impact of SCFAs on CFTR maturation, we performed Western blot analysis on whole cell lysates. Further, we tested SCFAs in primary nasal epithelial cultures from patients with CF homozygous for the F508del mutation.

Our results showed relatively small standalone effects but strong synergy between SCFAs and ETI in restoring F508del-CFTR function. The combination therapy enhanced CFTR-mediated currents fourfold compared to ETI alone in CFBE41o cells. Additionally, propionate and valerate demonstrated similar synergism, increasing CFTR-mediated currents approximately twofold when combined with ETI. Preliminary data in nasal epithelial cultures show an increase in CFTR-mediated ion transport through the combination of ETI with either butyrate or valerate. These findings show that SCFAs enhance the ETI-mediated pharmacological restoration of F508del-CFTR chloride channel function, thereby identifying a further putatively beneficial effect of commensals' metabolites. These findings help to explore an additional therapeutic approach to further improve F508del-CFTR function.

Combined stem cell and predictive models reveal flavin cofactors as targets in metabolic liver dysfunction

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Drug discovery for metabolic dysfunction-associated steatotic liver disease (MASLD) is challenging due to inadequate models and untargeted drug screenings. We combine human stem-cell-based modeling with computed predictions identifying flavin pathways as MASLD targets. First, we compounded injuries in human iPSC-derived hepatocytes (iHeps) culminating in metabolic crisis. Adipo-, Myokines and leukocyte trans-well co-culture had the most impact on metabolic deterioration, causing transcriptional reprogramming similar to F4 fibrosis stages of human hepatic MASLD samples (n = 206). A three-pronged machine learning-powered drug repurposing approach using human MASLD liver transcriptomes tailored to metabolic crisis in hepatocytes predicted flavin adenine dinucleotide (FAD) to reverse the disease phenotype. We validated flavin-pathway dysregulation in vivo through proteomics of a pediatric (n = 70) and transcriptomics of an adult (n = 508) cohort. To functionally validate therapeutic flavin manipulation, we titrated effective FAD concentration to 50 µM, rescuing steatosis, mitochondrial dysfunction, and reversing pathologic transcriptional programs such as fibrogenic signaling which we validated using multicellular liver organoids. Further, flavin-manipulation blunted MASLD-associated activation of primary monocytes and T-cells in vitro. To identify orally available flavo-active compounds, we queried similar network topologies, predicting e.g., norleucine and aspirin which rescued mitochondrial dysfunction in doses similar to therapeutic in vivo concentrations. We amplified flavin-effects via genetic stimulation of mitochondrial biogenesis by using open reading frame overexpression of *PPARGC1A*. Our study demonstrates how integrating stem cell-derived disease modeling with computed drug prediction can expedite therapeutic discovery in a human centric manner.

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Modelling AKI in vitro using Tubuloids generated from urine derived primary Renal Epithelial Cells

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Acute kidney injury (AKI) results in the episodic loss of kidney function. AKI occurs in 1-30% of hospitalised patients, and 30-60% in the critically ill. Of these, 20-50% develop progressive chronic kidney disease (CKD) and 3-15% reach kidney failure. Interventions that can prevent or reverse AKI and/or maladaptive fibrotic processes are currently lacking. This is in part thanks to failures with the presently available experimental AKI models; where one-fifth of all lead therapeutics from pre-clinical testing fail during clinical trials due to nephrotoxicity.

Tubuloids are 3D renal-tubule organoids generated from primary RECs that form continuous, polarised, intact epithelia that emulate the functions of discrete tubule segments. Using tubuloids generated from urine isolated RECs, we have modelled several features of kidney injury. To emulate ischaemia-reperfusion (IR), one of the most common AKI contributors, tubuloids were exposed to hypoxia (16 h at 1% O₂, glucose free media) followed by normoxia (20% O₂, tubuloid growth media). At the 3h timepoint, RNA sequencing revealed upregulations expression profiles related to oxidative stress that have resolved by the 24h timepoint to be replaced with a pro-inflammatory profile. To mimic drug induced AKI, gentamicin, a common antibiotic and nephrotoxin, was applied to tubuloids for 24h, producing marked increases pro-fibrotic character. Loss of viability and apoptotic induction were seen in all “injured” tubuloids. Interestingly, when compared to other RNA sequencing data sets from human kidney biopsies and urine, the gentamicin treated tubuloids showed strong correlations to in vivo human AKI. Untreated tubuloids meanwhile showed good correspondence to healthy kidney tissue, while the IR treated tubuloids clearly diverged. Together we have shown that relevant injury stimuli can induce AKI like profiles in tubuloids. Given their ease of handling, low cost, personalisable and human character, this system may prove an important alternative to current nephrological testing, especially in combination with recent differentiation strategies.

Investigation of bioactive surface-functionalized multilayer nanoparticles with a 3D ex vivo human wound healing model

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Physiological differences between animal and human wounds undermine the predictive value of *in vivo* studies for assessing therapeutic efficacy and safety. Meanwhile, cell cultures fail to recapitulate the complex architecture and immune microenvironment of human wounds. Therefore, our research group is dedicated to developing 3D wound healing models based on ex vivo human skin that more accurately reflect the human wound microenvironment.

Here, we applied a 3D wound healing model to investigate the biological performance of engineered nanoparticles fabricated via the layer-by-layer technique (LbL-NPs). We first used human keratinocytes (HaCaT cells) to investigate the *in vitro* cellular internalization behavior of LbL-NPs. Interestingly, we found that polysaccharides in the outer layer enhanced NPs cellular uptake. Using confocal microscopy and flow cytometry, we confirmed the efficient cellular uptake of LbL-NPs. The scratch assay further showed that surface-functionalized LbL-NPs enhanced HaCaT cell migration, indicating their pro-healing potential.

Building upon these findings, we employed the ex vivo wound model to assess the biological performance of LbL-NPs. (i) LDH assays indicated excellent biocompatibility; (ii) ELISA of inflammatory cytokines IL-6 and IL-8 revealed no evidence of an inflammatory cascade response; (iii) histological analyses, including hematoxylin-eosin (H&E) and immunofluorescence staining of re-epithelialization (keratin-17) and re-vascularization (CD31) markers, further confirmed that LbL-NPs and specifically those coated with hyaluronic acid promoted re-epithelialization and accelerated neovascularization within the wound area.

Collectively, these results demonstrate that the 3D wound healing model not only recapitulates the structural and functional characteristics of the native wound microenvironment but also provides a platform for histological and molecular-level assessments of nanomaterial safety and therapeutic efficacy. This approach addresses key limitations of traditional 2D culture systems and animal models in terms of physiological relevance and immunological predictability, offering a feasible strategy for the preclinical evaluation of developed wound healing formulations.