Abstract Book

CAOS
Conference on Advancements in Organoid Sciences on January 30th + 31st 2025

Generating patient-derived lung normal and tumour organoids from different cells of origin and with different mutational background

Vittorio Barbè, *The Francis Crick Institute*

The TRACERx program investigates tumour evolution in early-stage respectable non-small cell lung cancer (NSCLC). At the Swanton laboratory, we generate bronchial and alveolar organoids from normal and tumour regions of lung tissue to model and understand these processes. Tissue samples, collected immediately post-surgery at University College London Hospitals (UCLH), are processed at the Francis Crick Institute. Samples are dissociated into single cells and either immediately processed or cryopreserved for future use. Organoids are cultured in GELTREX within specialized media, with formation taking approximately three weeks. Success rates are 59% for tumour-derived organoids and 74% for normal tissue.

Quality control at passage 3 involves histological and immunohistochemical staining (H&E, CK5, TTF1, p63), short tandem repeat analysis, and tests for mycoplasma and human viruses. Notably, organoids generated from different regions of the same patient often exhibit morphological variability, influencing protocol adaptations and co-culture dynamics. Select samples undergo whole-genome sequencing to compare the genomic landscapes of organoids and their original tumours. For instance, specific organoid subtypes display dominance in co-culture systems, necessitating adjustments to improve reproducibility and performance.

Current efforts focus on generating alveolar organoids from primary lung tissue, developing models from tumours with specific driver mutations, and creating organoid lines representing diverse ethnic backgrounds. With these advanced aims, we aim to elucidate tumour evolutionary mechanisms and guide the development of effective therapeutic strategies through patient-derived, cellular models.

Abstract for the CAOS 2025 Conference on Advancements in Organoid Sciences

Modeling Brain Tumor Treatment: Comparing Single and Hypofractionated Carbon Ion Therapy in Organoid Cultures

Tamara Bender[1], Esther Schickel[1], Leon Kaysan[1], Simone Hufgard[2], Margot Mayer[2], David Grosshans[3], Marco Durante[1], Insa S. Schroeder[1]
[1]GSI Helmholtzzentrum für Schwerionenforschung GmbH, Planckstraße 1, 64291 Darmstadt, Germany
[2] Technische Hochschule Aschaffenburg, University of Applied Sciences, Würzburger Str. 45 63743 Aschaffenburg, Germany
[3]The University of Texas MD Anderson Cancer Center, Houston, Texas, United States

Radiotherapy is crucial in managing brain tumors, particularly when no effective alternatives exist. However, challenges in tumor targeting persist due to the lack of appropriate models. Here, we used a tumor model comprising of brain organoid slices generated from human induced pluripotent stem cells, cultured alone or with genetically modified brain tumor-like cells ($GFP^+/cMYC^{high}$), to study the effects of carbon (C)-ion irradiation a state of the art technique that may provide improved disease control.

We compared the outcomes of a single 15 Gy versus hypofractionated (3x7.5 Gy) dose, both delivering the same biologically effective dose. Cell death, gene, and protein expression were assessed at 7, 20, and 70 days post-irradiation.

$GFP^+/cMYC^{high}$ cells showed oncogene overexpression, tumor suppressor gene downregulation, increased proliferation, and invasiveness, leading to overgrowth of the organoid slices. A single 15 Gy dose caused a transient increase in lactate dehydrogenase (LDH), a marker of necrosis, which was absent after the hypofractionated regimen. No significant changes in LDH levels were observed in normal organoid slices at later time points. Tumor cell killing was confirmed by reduced GFP^+ cells and decreased cMYC mRNA expression. At 70 days, regrowth of tumor-like cells occurred after the single dose but not after hypofractionated irradiation.

Brain organoid slices co-cultured with tumor-like cells are an effective model for radiotherapy studies. Hypofractionated C-ion therapy reduces normal tissue toxicity and more effectively prevents tumor regrowth compared to a single high dose, suggesting it may offer a better balance between efficacy and tissue preservation in brain tumor treatment.

Title: Midbrain organoid model for Parkinson's research

Authors: Ankush Borlepawar, Parvana Hajieva

Affiliation: Cellular adaptation and bioenergetics group, Institute for Translational Medicine (ITM) Medical Faculty of the Medical School Hamburg

Abstract

Introduction: With late onset, accelerated pathology and non-availability of treatment options, neurodegenerative disorders like Parkinson's present unique challenges for clinical research. The investigation of the molecular mechanisms leading to the degeneration of dopaminergic cells is important in order to identify early therapeutic targets. Several *in vitro* and *in vivo* models are employed for the Parkinsonian research worldwide. 3D brain organoids represent an attractive model as they represent a special brain region and is derived from human cells.

Methodology: Briefly, we established a 3D organoid model according to the protocol of Pasca lab (Stanford University, USA) and employed STEMdiff Midbrain organoid differentiation kit and Maintenance kit from STEMCELL Technologies (Vancouver, Canada). AggreWell^{TM}800 plate was used to create the embryonic bodies from human pluripotent stem cells that were later transferred to 6-well plates for subsequent expansion, differentiation into midbrain organoids and maintenance for up to 135 days. The differentiated midbrain organoids (d60 and d120) were then fixed, cryopreserved, sliced and subjected to IHC using primary neuronal antibodies and fluorescent secondary antibodies. The fluorescence micrographs were acquired using a confocal microscope (Leica, Germany) at different magnifications.

Conclusion: The stronger presence of nuclear DAPI and neuroepithelial SOX2 in the central part of midbrain organoids compared to edges points to a continuous dividing core and higher peripheral maturation. Immature neuronal marker Tuj1 appears throughout the organoid, while mature neuronal markers NeuN and MAP2 display a stronger presence towards the periphery. This observation is further reinforced, as mature dopaminergic markers like GIRK2 and FOXA2 are predominantly present at the organoids' periphery. Additionally, the glial/astrocyte marker GFAP is scarcely present, underscoring that the organoids consist of a highly neuronal population. Collectively, with a healthy population of neurons, low glial interference and a high peripheral percentage of mature dopaminergic neurons, the midbrain organoid model established in our lab presents a promising model Parkinson's research.

Title: Advances in understanding in vitro Dorsal Forebrain Organoid models.

Authors: Tomas Fanutza[1], Maximilian Borgmeyer[2], Nina Wittenmayer[2], Christian Wozny[1].

Affiliations:

[1] Laboratory of Neuronal Diversity and Microcircuits in Health and Disease, Institute for Molecular Medicine, MSH Medical School Hamburg, Germany.

[2] Institute for Translational Medicine (ITM), MSH Medical School Hamburg, Germany.

Abstract:

Over the past decade, significant advances in neuroscience research have been made by novel and pioneering 3D in-vitro cultures now referred to as neural organoids, which are derived from induced human pluripotent stem cells (hiPSCs). These organoids closely replicate key aspects of human brain structure and function exhibiting sophisticated cellular organization, layer formation and mature gene expression profiles. This innovative technology is instrumental for studying neural development, mechanisms of neurodegenerative disorders and drug responses. Moreover, patient-specific organoids could serve as invaluable in vitro models for exploring therapeutic interventions. Our laboratory employs a variety of assays to investigate cerebral organoids to facilitate a more comprehensive investigation of cerebral organoids. Using patch-clamp electrophysiology, we measure synaptic activity and related electrical properties of neuronal organoid cells. To explore the heterogeneity of neuronal populations and decipher synaptic protein composition, we apply immunostaining and advanced microscopy techniques. We aim to track the emergence and maturation of active, functional neuronal networks as well as to assess their long-term viability. Overall, these sophisticated 3D models will foster our understanding of how neurons develop connections in the human brain and will be useful for studying common neurological disorders such as depression, autism, and Alzheimer's disease.

Poster Abstract for CAOS conference 2025, Hamburg

Title: Cultivation of cortical organoids

Authors: Katharina Geier, Laura Diener-Hölzl, Christine Kuang, Maik Ködel, Matthias Eder, Ruhel Ahmad, Elisabeth Binder

Max Planck Institute of Psychiatry, Munich, Germany.

Human brain development is a complex process that follows a pattern common to all mammals, but also includes features unique to humans. Understanding human neurodevelopment is crucial, as environmental and genetic risk factors can influence brain development as early as the prenatal stage, potentially increasing the likelihood of psychiatric disorders later in life.

Human cerebral organoids derived from iPSCs offer a unique in vitro model for studying early neurodevelopment while preserving the human-specific characteristics of cerebral development. They are widely used to study various psychiatric disorders. However, challenges emerge in the cultivation of brain organoids, including reproducibility issues and significant variability among organoids as well as late maturity.

The objective of this study is to establish a guided protocol starting from pluripotent stem cells, to grow cortical organoids as consistently and reliably as possible.

Here, we demonstrate the reproducible formation of cortical organoids, subjected to varying morphogen treatments at distinct early stages of neurogenesis. We performed bright-field microscopy, qPCR analysis and tissue clearing with 3D immunohistochemical staining of organoids at days 24 and 55 of cultivation to analyze the cellular composition of organoids cultivated under eight distinct conditions.

We saw that enhanced activation and inhibition of early neurodevelopmental pathways altered the morphological growth and cellular composition of the organoids.

To conclude, the careful selection of suitable cell lines and the optimization of culture conditions through the precise addition of morphogens at optimal concentrations and time points are essential to achieve reproducible and reliable cortical organoid models to study human neurodevelopment.

Reliability of high-quantity human brain organoids for modeling microcephaly, glioma invasion and drug screening

Jay Gopalakrishnan, *Institute for Human Genetics*
Laboratory for Centrosome and Cytoskeleton Biology, Universitätsklinikum Jena

Brain organoids offer unprecedented insights into brain development and disease modeling and hold promise for drug screening. Significant hindrances, however, are morphological and cellular heterogeneity, inter-organoid size differences, cellular stress, and poor reproducibility. Here, we describe a method that reproducibly generates thousands of organoids across multiple hiPSC lines. These High Quantity brain organoids (Hi-Q brain organoids) exhibit reproducible cytoarchitecture, cell diversity, and functionality, are free from ectopically active cellular stress pathways, and allow cryopreservation and re-culturing. Patient-derived Hi-Q brain organoids recapitulate distinct forms of developmental defects: primary microcephaly due to a mutation in CDK5RAP2 and progeria-associated defects of Cockayne syndrome. Hi-Q brain organoids displayed a reproducible invasion pattern for a given patient-derived glioma cell line. This enabled a medium-throughput drug screen to identify Selumetinib and Fulvestrant, as inhibitors of glioma invasion *in vivo*. Thus, the Hi-Q approach can easily be adapted to reliably harness brain organoids' application for personalized neurogenetic disease modeling and drug discovery.

Human cerebral organoids as a model to study the interaction of glioblastoma with resident glial cells

S. Kalpazidou[1], P. Moysidou-Tsiorva[1], B. Özbaykent[1], E.R. Shelton[3], M.Frischmann[3], S.Chakraborty[5], J. Spalevic[1], C.Delbridge[5], N.Pfarr[5], J. Schlegel[5], F. Serwane[2,3,8], M. Ninkovic[1], A.Wagner[10], J. Ninkovic[1,2,7,9]

[1] Cell Biology and Anatomy, LMU, Munich, Germany
[2] Graduate School Systemic Neurosciences, LMU, Munich, Germany
[3] Faculty of Physics and Center for NanoScience, LMU, Munich, Germany
[4] German Center for Neurodegenerative Diseases (DZNE), Munich, Germany
[5] Institute of Pathology, Faculty of Medicine, TUM, Munich, Germany
[6] Institute of Computational Biology, Helmholtz Zentrum Munich, Munich, Germany
[7] Biomedical Center Munich (BMC), Faculty of Medicine, LMU, Munich, Germany
[8] Munich cluster for Systems Neurology SYNERGY, LMU, Munich, Germany
[9] Institute of Stem Cell Research, Helmholtz Zentrum Munich, Munich, German
[10]Department of Neurosurgery, Klinikum rechts der Isar, Technical University Munich

Glioblastoma multiforme (GBM) is one of the deadliest brain cancers, characterized by complex interactions with the diverse resident brain cells that form the GBM niche. This niche plays a pivotal role in driving GBM progression and determining patient prognosis. However, the complexity and functional redundancy of the cells within the GBM niche have hindered a precise understanding of their individual roles, particularly those of glial cells. To address this, we developed human cerebral assembloids with innate microglia and macroglia, subsequently transplanting them with human GBM cells to model tumorigenesis. Our model replicates key features observed in patients, including a distinct tumor core, peritumoral, and tumor-free areas, tumor stiffness comparable to patient data, GBM-associated methylation patterns in cancer cells, and tumor-induced glial reactivity at both transcriptomic and protein levels. Additionally, this system is modular, enabling coculturing with various differentiated cell types, such as monocytes, to investigate immune-tumor interactions. By leveraging iPSC-derived human cerebral assembloids, this innovative in vitro platform provides a powerful tool to study GBM-induced cellular reactivity and its potential role in tumor progression.

Title: The Analysis of Neurodevelopmental Disorders by the Generation of 3D Cerebral Organoids Mimicking the Human Cerebral Development

Authors: Sohana Islam Khan, Anne-Sophie Gimpel, Bernadette Harwardt, Thomas Hollemann, ⋆**Matthias Jung.**

Corresponding author: Matthias.jung@uk-halle.de

Affiliation: Martin-Luther University Institute of Physiology and Chemistry department.

Abstract

Introduction: The advancing fields of organoid and embryonic research offer unprecedented insights into the complexity of human development, disease modeling, and potential therapeutic strategies. This study explores cutting-edge methodologies in neuroscience, focusing on organoids as transformative tools for understanding neurodegeneration, various developmental disorders like which organs tissues or cells are affected and how it associated with aging. Organoids, derived from pluripotent stem cells, mimic the structure and function of brain regions, enabling researchers to investigate conditions like Alzheimer's, dementia, and schizophrenia. Analysis remains difficult because special techniques are needed for the analysis of organoids.

Methods: We generated cerebral organoids from human iPS cells, analyzed them by gene expression and verified localization of proteins by immunofluorescence analysis.

Results: This research emphasizes the limitations of traditional 2D cultures and animal models, advocating for advanced 3D systems that integrate cellular microenvironments with patient-specific genetic backgrounds. We successfully generated cerebral organoids mimicking layering of the human cerebral cortex in part. We also have proofed the presence of differently specified cellular layers by the presence of certain mature and functional markers. The development of cerebral organoids was shown at ~35 days by specific neurodevelopmental markers (Pax6, Tubb3, Olig2, FoxG1, and ASCL1).

Conclusion: Together, these data suggest a successful generation of cerebral organoids, which replicate a certain brain layer morphogenesis. Further applications may include organoid intelligence (OI) as a paradigm for biological computing. Further applications ranging from high-throughput drug screening to space research, this work underscores the transformative potential of organoids in bridging experimental gaps.

Title: Advancing and modulating human heart-forming organoids (HFOs) for drug testing

Authors:

Felix Kleemiß[1], Liam Wilson[1], Dr. Miriana Dardano[1], Jana Teske[1], Annika Franke[1], Laura Ober[1], Lika Drakhlis[1], Robert Zweigerdt[1]

[1]Medical School Hannover (MHH) Leibniz Research Laboratory for Biotechnology and Artificial Organs (LEBAO)

Abstract:

Although their numbers were drastically decreased since the 1970s, animal models still play a major role in life sciences and drug development, despite having numerous drawbacks such as high costs and low predictive capabilities for the human organism. Therefore, organoids derived from human pluripotent stem cells (hPSCs) play a key role in the replacement of animal models in disease modelling and drug screening. The heart-forming organoid (HFO) system is a hPSC-derived pre-vascularized and patterned multi-tissue organoid model that closely resembles early heart and foregut development. To establish HFOs as a platform for drug screening, we exposed them to FDA-approved antiangiogenic chemotherapeutic compounds. Their effect on the vascular network and other tissue types within the HFO was evaluated by flow cytometry (FC) and whole-mount immunofluorescence staining (WMS). Confocal images were processed using Python-script and analysed using AngioTool, which allowed a quantitative analysis and characterisation of the vascular network. Treating HFOs with Axitinib and Cabozantinib in a clinically relevant concentration range (c_{max}) resulted in a strong antiangiogenic effect without decreasing the viability of the organoids. Axitinib did not show any effect on the cardiac and pluripotent compartment of the HFO while altering the expression pattern of endodermal surface markers. These preliminary results highlight the HFOs' potential as a robust drug screening platform to test antiangiogenic compounds while simultaneously investigate possible off-target effects on cardiac and foregut tissues.

Liver organoids in metabolic assays

Victoria Zopf[1], Zina Riahi[1], Kristijan Skok[2], Karoline Lackner[2], Daniela Kniepeiss[3], Robert Karitnig[3], Andreas Prokesch[1], Jelena Krstic[1]

[1] Cell Biology, Histology and Embryology; Gottfried Schatz Research Center; Medical University of Graz, Austria

[2] Diagnostic and Research Institute of Pathology; Medical University of Graz, Austria

[3] Division of General, Visceral and Transplant Surgery; Medical University of Graz, Austria

The liver is a key metabolic organ that rapidly responds to nutrient availability and metabolic changes. *In vitro* models for studying liver metabolism, including conditions such as hepatocellular carcinoma (HCC), have traditionally relied on cancer-derived cell lines. However, the development of organoid isolation techniques from patient liver samples has introduced long-term models with preserved metabolic activity.

We utilized liver- and HCC-derived organoids to investigate the effects of oxidative phosphorylation (OXPHOS) inhibitors in combination with nutrient deprivation (ND) on cell viability. Our findings indicate that ND enhances the efficacy of certain inhibitors. To better replicate ND conditions, we are developing a fasting-like medium to test in organoid models. OXPHOS inhibition was assessed using live-cell imaging and Seahorse assays.

Furthermore, to model liver steatosis, we loaded liver organoids with palmitic acid (PA). The PA-loaded organoids were subsequently treated with a specific bile acid. Mitochondrial activity and the expression of metabolic genes were then measured to assess the effects of these treatments. The bile acid significantly reduced lipid accumulation in PA-treated organoids and significantly increased mitochondrial respiration in both, PA and non-PA treated conditions. In addition, the bile acid treatment resulted in similar gene expression pattern as observed *in vivo* as well as in HCC-derived cell line.

Taken together, our results demonstrate that liver organoids are valuable models for functional metabolic studies, offering potential applications in drug testing and molecular signaling research.

Generation of triple assembloids to model cortico-striatal and nigro-striatal connectivity for Parkinson's disease

Pauline Lachal[1], Christiane Zhu[1], Gabin Devely[1], Evodie Lassalle[1], Helena Winterberg[1], Benjamin Galet[1], Charlotte Deleuze[2], Nelson Rebola[3], Olga Corti[1], Hélène Cheval[1], Philippe Ravassard[1]
[1]Molecular Physiopathology of Parkinson's Disease Group, P. Ravassard, JC. Corvol,
[2] Electrophysiology core facility, Patis Brain Institute
[3]Cellular mechanisms of sensory processing Group, N. Rebola
Paris Brain Institute (ICM)/CNRS UMR 7225/INSERM 1127/ Sorbonne University, Paris, France

Parkinson's Disease (PD) is characterized by the degeneration of dopaminergic (DA) neurons in the substantia nigra, resulting in motor deficits. These neurons project to the striatum, where they modulate cortical motor integration by medium spiny neurons (MSNs). Traditional animal models using neurotoxins or transgenic mutations offer insights into PD but fail to fully replicate the human disease, limiting treatment development.

To improve PD modelling, we developed a 3D human organoid system derived from induced pluripotent stem cells (iPSCs). This model integrates ventral midbrain organoids (VMO) representing DA neurons, ventral forebrain organoids (VFO) for striatal MSNs, and dorsal forebrain organoids (DFO) for cortical neurons, recreating key aspects of basal ganglia circuitry.

We confirmed DA neuron differentiation in VMOs, MSN markers (DARPP32) and GABA-expressing cells in VFOs, and glutamatergic cortical layers in DFOs by day 90. Enhanced maturation of DA neurons and cortical organization were observed, with DA and glutamatergic projections observed in VFO, in the vicinity of the MSNs.

Preliminary results using PRKN knock-out (KO) iPSCs, a gene linked to autosomal recessive PD, demonstrated altered calcium activity in VFO-VMO assembloids. These findings highlight the potential of this model to unravel mechanisms of DA neuron degeneration and basal ganglia circuit development in PD.

CORTICAL IMMUNOCOMPETENT ASSEMBLOID AS THERAPEUTIC MODEL FOR ALZHEIMER'S DISEASE

Evodie Lassalle 1,2, Benjamin Galet 1, Clément Daube 1, Anne Lorenz 1, Stéphanie Bigou3, Ludovic Collin 2, Olga Corti 1, Myriam Ly-Le Moal 2 et Philippe Ravassard 1

1 Equipe Ravassard-Corvol, Paris Brain Institute/CNRS UMR 7225/INSERM U1127/Sorbonne Université, Paris, France;

2 Institut Roche, Paris, France;

3 Plateforme ICV-3C Paris Brain Institute;

A-bêta peptide (Aβ) is a neuronal protein that is a key factor in the development of Alzheimer's disease, since its abnormal aggregation leads to the formation of insoluble protein structures known as β-amyloid plaques which are involved in the neurodegeneration characteristic of Alzheimer's disease (AD).

GWAS studies have identified Triggering Receptor Expressed on Myeloid cells 2 (TREM2) as a gene associated with AD. The TREM2 receptor is predominantly present in microglia and promotes their survival, activation and participates in neuroprotection, whereas a deficiency in this gene leads to a decrease in microglia viability and proliferation, as well as a reduction in the removal of β -amyloid plaques characteristic of AD.

The focus of this project is the generation of immunocompetent cortical assembloids originating from AD patient-derived cells or their isogenic healthy control, that would recapitulate main hallmarks of AD, including β-amyloid plaques. The assembloid model aims to mimic human cortex development with the generation of organoids modeling the dorsal region of the forebrain (DFO, production of excitatory neurons) or the ventral zone of the forebrain (VFO, production of inhibitory interneurons). In a second step, these two regions are fused to form assembloids. Eventually, the immune component will be added using iPS cell-derived microglia. Such models would provide a functional platform to implement and study the contribution of WT and/or TREM2 mutant microglia in the development of the pathology with a special focus on mechanisms leading to reduction of the amyloid load in the AD models.

Title
Utilising a clinostat-based system to advance in vitro liver fibrosis modelling and therapeutic investigations.

Authors
Karoline Lindgård Mikkelsen

Krzysztof Wrzesinski

Helle Sedighi Frandsen

Adelina Rogowska- Wrzesinska

Affiliations
University of Southern Denmark, CelVivo

Abstract
Liver fibrosis, characterized by excessive extracellular matrix deposition, is a hallmark of chronic liver injury. This study aimed to develop an advanced *in vitro* liver fibrosis model using a clinostat-based system for therapeutic investigations. Human hepatic stellate cells (LX-2) were cultured into spheroids within a ClinoReactor system and treated with transforming growth factor-beta 1 (TGFβ1) to induce a fibrotic phenotype. Biomarker production, including clinically relevant peptides such as PRO-C1, PRO-C3, and FBN-C, was analyzed over time to assess fibrosis progression. Results demonstrated that TGFβ1 treatment elevated mRNA expression of fibrotic markers, including COL1A1, COL3A1, COL6A1, ACTA2, and TIMP1, reflecting hepatic stellate cell activation and increased collagen synthesis. Therapeutic intervention with Nintedanib, an anti-fibrotic agent, significantly reduced the expression and secretion of COL1A1, COL3A1, and COL6A1 peptides, indicating a decrease in fibrotic activity and biomarker production. Biomarker levels returned to control conditions after 5–12 days of treatment, further validating the model's utility in recapitulating liver fibrosis. The clinostat-based system provided a dynamic microenvironment that enhanced spheroid formation, ensuring the production of clinically relevant fibrotic biomarkers. This *in vitro* platform enables precise monitoring of fibrogenesis and therapeutic efficacy, advancing preclinical anti-fibrotic drug testing. Overall, this model bridges the gap between current *in vitro* methods and clinical relevance, offering a robust tool to study liver fibrosis pathophysiology and evaluate novel treatments.

ABSTRACT

Animal free synthetic biomimetic fibrous scaffolds for the setup of 3D small intestinal mucosa *in vitro* models

Sarah Blümel [1], Christina Fey [2], Tobias Weigel [2], Bastian Christ [2], Maximiliane Wußmann [2], Jörn Probst [2], Daniela Zdzieblo [1,2]

[1]University Hospital Würzburg, Department of Tissue Engineering and Regenerative Medicine (TERM), Würzburg, Germany; [2]Fraunhofer Institute for Silicate Research (ISC), Translational Center Regenerative Therapies (TLC-RT), Würzburg, Germany.

The stromal part is a key element of current tissue equivalents as an alternative for animal experimentation. Due to the lack of synthetic alternatives, most researchers rely on animal derived materials such as collagen [1]. However, besides ethical concerns, these scaffolds show batch-to-batch variances together with increased costs. Suitable synthetic 3D scaffold materials have to fulfill several challenges, like being bioactive, highly porous or biomimetic [1]. Here we present the generation of highly porous fibrous scaffolds with subsequent cellular biologization, resulting in synthetic-based 3D scaffolds used for a wide variety of current tissue models such as the intestine, consisting of connective tissue and epithelium.

A conventional electrospinning process was modified by the combination with porogens, resulting in a highly porous scaffold. For the setup of small intestinal *in vitro* test systems, they were seeded with small intestinal stromal tissue cells (hiF) for 2 weeks, followed by primary enteroids seeded as single cells on the respective scaffolds, forming a confluent cell layer after 10 days [2,3].

Our newly developed scaffolds allow cellular migration of primary human fibroblasts together with a dynamic rearrangement of the fibers, which results in homogenously populated scaffolds and tissue specific ECM components. The combination with human intestinal epithelial cells generated physiological and functional models of the intestine by a representative cellular composition, barrier integrity as well as cell polarization.

Taken together, our scaffold material enables the generation of a platform technology, which can address a diversity of current tissue models and enables replacement of animal derived materials.

References

[1] Weigel, T., Malkmus, C., Weigel, V., et al. (2022). ***Adv. Mater. 34***, 2106780. doi: 10.1002/adma.202106780
[2] Sato, T., Stange, D. E., Ferrante, M. et al. (2011). ***Gastroenterology*** *141*, 1762-1772. doi: 10.1053/j.gastro.2011.07.050
[3] Schweinlin, M., Wilhelm, S., Schwedhelm, I. et al. (2016). ***Tissue Engineering Part C Methods*** *22*, 873-883. doi: 10.1089/ten.TEC.2016.0101

Complex mechanisms of cortical malformations: interaction between genetic predisposition and prenatal hypoxia

Indra Niehaus[1], Pia Reimann[1], Jan Fischer[2], Evelin Schröck[3], Michael Heide[4], Nataliya Didonato[1]

[1]Department of Human Genetics, Hannover Medical School, Hannover, Germany
[2]Institute for Clinical Genetics, Technische Universität Dresden, Dresden, Germany
[3]Institute for Clinical Genetics, Technische Universität Dresden, National Cancer Center (NCT), Dresden, Germany
[4]German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Introduction:
Malformations of cortical development (MCD) have a complex etiology and exhibit significant heterogeneity, resulting in highly variable clinical pictures among affected individuals. Baraitser-Winter cerebrofrontofacial syndrome (BWCFF) associated with the missense variants in *ACTB* and *ACTG1*, is one of the demonstrative examples of the clinically variable MCDs. MCDs are a hallmark feature of BWCFF but show incomplete penetrance, raising the question of the potential modifying factors. We hypothesize that prenatal hypoxia might exacerbate MCD in BWCFF patients.

Methods:
Reprogramming of iPSCs from primary cells of two unrelated patients with ACTB:Arg196His variants (most frequent recurrent variant in BWCFF); differentiation into cerebral organoids (Lancaster protocol), cultivation under normoxic (21%) and hypoxic conditions (5% and 7%) with the subsequent characterization of the size and cellular composition.

Results:
As shown previously, BWCFF cerebral organoids are significantly smaller than the healthy controls under normoxic conditions. Growth under 5% hypoxic conditions was accompanied by expression of HIFα (Hypoxia-Inducible Factor) and led to considerably reduced size and cell number of both BWCFF and control organoids. However, the first experiment with 7% hypoxia only impacted the growth of BWCFF organoids, whereas control organoids resembled their normoxic counterparts.

Conclusion:
Our model supports that mild short-term prenatal hypoxia might contribute to the development of MCD in BWCFF patients. Cerebral organoids allow for testing of other maternal exposures, such as alcohol, medications, hyperthermia, etc., to explore gene environmental interaction in cortical development.

Newly developed kidney tubuloid model shows improved structural, compositional, and functional properties

Tomáš Pelikán[1#], Viktória Filipková[1,2], Roman Zachoval[1,2], Petr Heneberg[1]

[1]Third Faculty of Medicine, Charles University, Prague, Czech Republic

[2]Department of Urology, Thomayer University Hospital, Prague, Czech Republic

[#]Correspondence: tomas.pelikan@lf3.cuni.cz, +420 723 062 244

Introduction: Kidney tubuloids are a three-dimensional *in vitro* model of renal tubules derived from adult stem cells. Despite improved representation compared to classical two-dimensional cultures, more representative models are needed.

Aim: To optimize the kidney tubuloid model, focusing on morphological and functional similarity to the renal cortex.

Methodology: We have designed a new cell culture medium formulation and compared the properties of tubuloids grown in this medium with those grown in standard previously published media. We assessed the morphological representation of the renal cortex by monitoring the culture with light and confocal microscopy. We determined the compositional representation of the renal cortex by flow cytometry. To assess the function of the resulting tubuloids, we employed the analyses of fluorescently labeled dextran internalization and secretion of fluorescently labeled methotrexate.

Results: The new culture medium significantly changed the morphology of tubuloids from cystic to convoluted structures. This change was reproducible in ten patient samples and persisted throughout several early passages of the cultures. The success rate of formation of advanced morphology was 100% by passage #4. Flow cytometry showed an increased representation of LTL^+ cells. Confocal microscopy confirmed the presence of narrow methotrexate-enriched lumens. Cells surrounding the narrow lumina concentrated fluorescently labeled dextran in their apical poles.

Conclusion: The new formulation of kidney tubuloid culture medium increases this model's morphological, compositional, and functional capacity and thus provides a key step towards better representation of the renal cortex using adult stem cell-derived models.

Support: Czech Health Research Council project NU23-06-00045.

IMPAIRED INTESTINAL STEM CELL FUNCTION IS LINKED TO MITOCHONDRIAL DYSFUNCTION IN COLONIC ORGANOIDS DERIVED FROM PATIENTS WITH ULCERATIVE COLITIS

Luise Pötter[1#], Charlotte Bein[1#], Lea Christiansen[1], Annika Raschdorf[1], Jasmin Ostermann[2], Timo Gemoll[2], Christian Sina[1,3,4], Franziska Schmelter[1], Martha M. Kirstein[3], Stefanie Derer[1]

[1] Institute of Nutritional Medicine, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany.

[2] Section for Translational Surgical Oncology and Biobanking, Department of Surgery, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany.

[3] Department of Medicine I, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany.

[4] Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering (IMTE), Lübeck, Germany.

[#] equally contributed

Cell differentiation in the colonic crypt is driven by a metabolic switch from glycolysis in intestinal stem cells (ISC) to mitochondrial oxidative phosphorylation (OXPHOS) in differentiated epithelial cells. Goblet cell dysfunction, as consequence of mucosal energy deficiency caused by reduced mitochondrial OXPHOS, has been attributed to ulcerative colitis (UC) pathogenesis. We aimed to functionally characterize underlying mechanisms of energy deficiency in patient-derived organoids (PDOs).

Here, UC patients displayed a metabolic shift towards increased lipoprotein serum levels transporting high levels of polyunsaturated fatty acids but low levels of saturated or monosaturated fatty acids. Mitochondrial dysfunction in UC patients was also reflected by increased ketone body production. In functional studies, we identified loss of $LGR5^+$ ISC function in UC-PDOs, which could be linked to the observed dysregulated metabolism.

These data provide new insights into metabolically driven impairment of the ISC compartment in UC that may enable identification of novel targeted nutritional intervention strategies.

Simulation of heat stress in chicken intestinal organoids – establishing an *in vitro* model

J. Riedel[1], J. R. Aschenbach[1], S. Trappe[1], S. Amasheh[1], L. Droessler[1]

[1]Institute of Veterinary Physiology, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Introduction: Heat stress is known to alter the expression of tight junction (TJ) proteins in the intestine of chickens. This study aimed to establish an *in vitro* heat stress model using chicken intestinal organoids (CIO).

Methods: Intestinal organoids from jejunal crypts of 4-week-old male Cobb500 broiler chickens (n = 2-3) were cultivated in 24-well plates at 37°C. For the experiments, fresh pre-heated medium (37°C, 40°C, 43°C) was added and plates were transferred into similarly pre-heated incubators for a 20 min period. Afterwards, the CIO were post-processed with or without a subsequent 10 min recovery period at 37°C. Protein expression of Zonula occludens 1 (ZO-1) and occludin were analyzed by Western blot and immunocytochemistry. The localization of the respective TJ proteins was confirmed using a confocal laser scanning microscope. Statistical analysis was performed using *t*-test.

Results: In the absence of a recovery period, ZO-1 and occludin expression was not affected after 40°C ($P > 0.05$), while incubation at 43°C led to a significant decrease of both TJ proteins in comparison to 37°C ($P < 0.05$). A subsequent recovery phase after the exposure to 40°C led to a significant decrease of ZO-1 and an increased expression of occludin in comparison to 37°C ($P < 0.05$). In contrast, a heat challenge at 43°C with recovery did not influence the expression of ZO-1 and occludin ($P > 0.05$).

Conclusion: The current CIO model appears suitable to simulate heat stress *in vitro* and provides initial insights into its impact on TJ proteins in chicken intestine.

ABSTRACT for the CAOS 2025

30th and 31th January 2025 in Hamburg

Modeling tumor infiltration in human cerebral organoids

Schickel E (1), Bender T (1), Persigehl J (1), Mayer M (2), Durante M (1), Schroeder IS (1)

(1) GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany, (2) Aschaffenburg University of Applied Sciences, bioMEMS Laboratory, Faculty of Engineering, Aschaffenburg, Germany

Brain tumors are generally associated with a poor prognosis despite state-of-the-art treatment. In radiotherapy, one of the major challenges is the tumor invasiveness requiring large target volume margins. This inevitably burdens the adjacent normal tissue and can lead to severe side effects such as demyelination resulting in cognitive impairment. To investigate the interaction between tumor and normal tissue and the effects of radiotherapy on tumor infiltration, we generated an autologous 3D human brain tumor model.

To mimic brain tumor initiation and promotion *in vitro*, an overexpression of c-Myc, the most prominent oncogene in brain tumors, was induced in sporadic cells of cerebral organoids generated from human embryonic stem cells. This led to randomly distributed tumor-like foci with high inter-individual heterogeneity. After proliferation of these GFP-tagged c-Myc^{high}-tumor-like cells within the organoids, comparability between the individual oncogenic organoids was increased by isolation of the GFP^{+}/c-Myc^{high} cells, aggregating them into spheres and fusing these spheres with cerebral organoids of the same genetic background, containing neurons and glia cells. c-Myc^{high} cells showed high proliferative and invasive potential into the organoids, while X-ray irradiation resulted in a reduced number of infiltrating cells and a decreased infiltration depth.

The model introduced here mimics essential parts of *in vivo* tumorigenesis within a cellular network resembling the human cerebral cortex. Due to its physiological character, this brain tumor model is suitable for high throughput analyses of radiation-induced effects. It highlights the importance of cell-cell interactions between normal and tumorous tissues in response to radiotherapy.

Investigation of viral infection in human lung and brain organoids derived from patient samples and genetically engineered human induced pluripotent stem cells (iPSCs)

Kristina L. Schierhorn[1*], Saskia Westhoven[1*], Simon T. Herrmann[1*], Miriam Martens[2], Natalie Heinen[1,3], Sabrina Schumann[1], Tina Meyer[1], Mona Sophie Schmitz[4,5], Marc Steinmeyer[6], Christian Conze[6], Holm Zaehres[7], Yannick Brüggemann[3], Michaela Schedel[4,5], Pietro Scaturro[2], Stephanie Pfaender[1,8]

[1]Research Group Emerging Viruses, Leibniz Institute of Virology, Hamburg, Germany
[2]Research Group Systems Arbovirology, Leibniz Institute of Virology, Hamburg, Germany
[3]Dept. of Molecular and Medical Virology, Ruhr University Bochum, Bochum, Germany
[4]Department of Pulmonary Medicine, Translational Pulmonology, University Hospital Essen - Ruhrlandklinik, Essen, Germany
[5]Department of Pulmonology, University Medical Center Essen, Essen, Germany
[6]Technology Platform Microscopy and Image Analysis (TP MIA), Leibniz Institute of Virology, Hamburg, Germany
[7]Dept. of Anatomy and Molecular Embryology, Institute of Anatomy, Ruhr University Bochum, Bochum, Germany
[8]Universität zu Lübeck, Lübeck, Germany
* These authors contributed equally to this abstract.

ABSTRACT

The study of viruses and their effects on innate immune signalling in tropism-specific tissues has been a challenge for decades. The development of organoid models in recent years has opened new ways to study viral tropism and local innate immune signalling in authentic tissues at the same time.

We established different human organoid systems based on iPSCs or patient-derived primary cells to study viral infection within a tropism-specific environment. By using iPSCs, we have the possibility for genetic modification, including gene knock-in/knock-out and the introduction of reporter constructs. By using patient-derived primary cells, we can analyse the impact of existing conditions on viral infection and innate immune signalling.

Most viruses use specific host-cell-expressed surface proteins as entry receptors, requiring organoids to not only recapitulate organ morphogenesis, but also spatial expression of these proteins on target cells. To study respiratory viruses, we use protocols for lung apical-out and apical-in organoids. As respiratory viruses initially infect apical cells, we established a protocol to section living apical-in lung organoids, which enabled us to perform viral infection of these organoids. We are currently investigating the impact of highly pathogenic and seasonal coronavirus infection in lung organoids on innate immune signalling.

To study neurotropic viruses, we use cerebral organoids derived from genetically-modified iPSCs with an IFN-dependent GFP-reporter. In these organoids, we are currently investigating the impact of different neurotropic viruses on IFN signalling and brain development.

Collectively, these studies will deepen our understanding of virus infection and innate immune response to inform the development of new therapeutic strategies.

Correcting a common CRB1 mutation by RNA base editing using retinal organoids

Denise Sperlich[1*], Davide Fumagalli[2], Thorsten Stafforst[2], Stefan Liebau[1] and Kevin Achberger[1]

[1] Institute of Neuroanatomy and Developmental Biology, University of Tübingen, Germany

[2] Interfaculty Institute for Biochemistry, University of Tübingen, Germany

* E-mail: denise.sperlich@uni-tuebingen.de

Retinitis pigmentosa (RP) is an inherited retinal dystrophy leading to visual impairment and blindness. Many causative genes play roles in photoreceptor or retinal epithelial cell functions like phototransduction, the visual cycle and cell homeostasis. The Crumbs complex protein CRB1 has over 150 identified mutations, with *C948Y* being the most common, caused by a single base pair exchange from guanine to adenine. While various gene therapies are being explored, no effective treatments exist, and the development of *CRB1*-targeted therapies is complicated by the presence of multiple isoforms.

Classical gene therapy methods may modify or replace the mutated *CRB1* gene, but they carry significant risks. An alternative approach uses antisense oligonucleotides (ASOs) to harness endogenous A-to-I RNA editing enzymes, to correct G-to-A base pair exchanges, as for instance the *C948Y* mutation in *CRB1*. We employed a modified RNA editing approach called RESTORE (recruiting endogenous ADAR to specific transcripts for oligonucleotide-mediated RNA editing), successfully correcting the *C948Y* mutation in *CRB1* using hiPSC-derived Müller glia cells as an initial *in vitro* model. Transfection and electroporation of *CRB1*-targeting ASOs achieved up to 40% correction of the point mutation.

To evaluate ASO penetration in retinal tissue, we used retinal organoids derived from RP patients and healthy donors. Various transduction methods, including lipofection, electroporation and magnetofection, were tested on retinal organoids. Correction efficiency in organoids was lower than in 2D models, emphasizing the importance of using three-dimensional cell models. These findings pave the way for developing further RESTORE ASOs targeting inherited point mutations with unmet clinical needs.

Advancing the Functional Characterization of Hippocampal Organoids with High-Density Microelectrode Array Technology

[4]Elisa Monz, [1,2]Fabio Benfenati, [2,3]Anna Corradi, [1,3]Martina Servetti, [2,3]Bruno Sterlini

[1] Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Largo Rosanna Benzi 10, Genova 16132, Italy;
[2] IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, Genova 16132, Italy.
[3] Dipartimento di Medicina Sperimentale, Università di Genova, Viale Benedetto XV, 3, Genova 16132, Italy
[4] 3Brain AG, Huobstrasse 16, 8808 Pfäffikon, Switzerland

Neurodegeneration and impairments in the hippocampus are major contributors to neurological and psychiatric disorders, including Alzheimer's disease, epilepsy and schizophrenia. Hippocampal organoids replicate key aspects of the human hippocampus under physiological and pathological conditions, providing a scalable and human-specific platform for studying the molecular and cellular underpinnings of these disorders. Building upon the protocol developed by Sakaguchi, we established a long-term air-liquid interface culture paradigm. This approach minimizes necrosis and extends culture viability, facilitating enhanced maturation and enabling more accurate functional studies.

Functional assessments of hippocampal organoid network activity and connectivity using high-density microelectrode arrays (HD-MEA) have remained largely unexplored. To address this, we employed 3Brain's HD-MEA technology, leveraging 4096 electrodes to investigate the electrophysiological activity of hippocampal organoid slices. Baseline activity and pharmacological modulation were evaluated using 4-aminopyridine (4-AP), bicuculline (BIC), and tetrodotoxin (TTX). Spiking and bursting activities exhibited consistent and reproducible patterns, modulated by drug treatments, underscoring the robustness of this system for functional studies. Additionally, we analysed parameters related to functional connectivity, furthering insights into network dynamics and their relevance to psychiatric disorders.

Our findings underscore the potential of HD-MEA technology to advance the functional characterization of hippocampal organoids, providing a robust platform for unravelling disease mechanisms, refining drug responses, and advancing therapeutic innovations.

Title: Stable and Highly Active Synthetic Peptide Growth Factors: Alternatives to BDNF, Noggin, and Wnt3a for Organoid Development

Author: Bärbel Icheln[1], Kazuki Shigematsu[2], Kosuke Minamihata[2]

Affiliation(s): [1]Active Bioscience GmbH, Germany, [2]Peptigrowth Inc., Japan

Abstract:

Conventional growth factors (GFs) used in organoid development face several challenges, including lot-to-lot variability, potential contamination with biological impurities, low stability, and high manufacturing costs. To address these issues, PeptiGrowth Inc. has developed synthetic peptides that replicate the functions of conventional GFs while overcoming these limitations. Additionally, these synthetic peptides offer regulatory advantages due to their chemically defined nature.

In this symposium, we will feature 3 alternative peptide growth factors: PG-003, PG-004, and PG-008, which are synthetic peptides for Brain-Derived Neurotrophic Factor (BDNF), Noggin, and Wnt3a, respectively.

PG-003 exhibits similar activity to recombinant BDNF (rBDNF). Using Nerve Organoids, a proprietary system developed by Jiksak Bioengineering, PG-003 enhanced neural axon growth, producing longer and thicker axon bundles compared to rBDNF.

PG-004 inhibits the signaling of BMP4 and BMP7 families. PG-004 effectively induced the differentiation of lung progenitor cells from hiPSCs and facilitated the formation of lung alveolar organoids at levels comparable to those achieved with Noggin.

PG-008 is a heterodimeric peptide that binds Frizzled and LRP5/6 receptors and exhibits agonistic activity on β-catenin pathway of WNT signaling. It effectively demonstrated the differentiation of iPSCs into definitive endoderm with comparable efficiency to CHIR99021. Notably, while CHIR99021 often faces issues of cytotoxicity, PG-008 exhibits no cytotoxicity up to 10 μM, the highest concentration tested. This contributes to increased development efficiency for various types of organoids.

In this presentation, we will present the data on PG-003, PG-004, and PG-008, along with application data using those peptides as well as other synthetic peptide GFs.

Title:

Spatial Transcriptomics Reveals Cellular Complexity in Organoids

Authors:

Kevin Bredemeyer, Ziming Zhong, Ana Vila-Santa

Affiliation:

Biomarker Technologies (BMKGENE)

Abstract:

Organoids are revolutionizing biomedical research as 3D models of human tissue. Leveraging BMKGENE's spatial transcriptomics technology, we achieved high-resolution mapping of gene expression within organoid samples, uncovering cellular heterogeneity and tissue organization across resolutions ranging from 100μm to 5μm.

This analysis revealed diverse gene expression patterns and UMI counts across spatial regions, offering valuable insights into organoid development and function. These findings highlight the transformative role of spatial transcriptomics in advancing organoid research for drug discovery and disease modeling.

Title:
Monitoring cell health at scale in 3D matrix cultures is as easy as in 2D

Authors:
Marco Brockmann[1], Martin Engel[1], Lisa Belfiore[1], Christine Yee[1], Nami Kamura[1], Margareta Sutija[1], and Toru Hattori[2]

Affiliations:
[1] Inventia Life Science Operations Pty Ltd, Alexandria, NSW 2015, Australia
[2] SCRUM Inc, Tokyo 135-0014 Japan

Abstract:
Advanced three dimensional (3D) cell culture models are aiming to address the need for adequate cell-cell and cell-environment interactions to enable biologically relevant processes. While the uptake of simple and complex 3D cultures is growing, the hurdle of collecting rich and meaningful data at scale of such cultures remains. Here, we describe a workflow for generating such advanced cell cultures with the RASTRUM™ platform, and common 2D cell culture assays for their suitability to assess core biochemical functions in medium-high throughput 3D cell cultures.To establish and validate the workflow, we treated the 3D synthetic hydrogel cultures containing glioblastoma cells (U87), hepatocytoma (HepG2, LX-2), or adenocarcoma (MCF-7) cells with either the histone deacetylase inhibitor Panobinostat or the tubulin-targeting chemotherapy drug Paclitaxel. We show that physiologically relevant tissue functions can readily be quantified in a high-throughput manner across these different cancer types, and five assay modalities, including high content imaging.

MO:RE Lab Platform: A New Automated Device to Standardize Organoid Culture

Júlia Vallverdú Ginès[1], Kim Krieg[2], Ole Pleß[2], Lukas Gaats[1]
[1]Mo:re GmbH, Hamburg, Germany - [2]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Hamburg, Germany

Human cortical organoids derived from induced pluripotent stem cells (iPSCs) represent an important *in vitro* system for disease modeling and drug screening. The applicability of organoids for high-throughput assays, however, requires automated workflows that enable a robust and homogeneous organoid production. We therefore developed the automated Mo:re lab platform to standardize organoid culture. In this study, we demonstrate the use of Mo:re's lab platform to support the production of cortical organoids in screening-compatible 96-well plates, following the STEMDIFF Dorsal Forebrain Organoid Differentiation Kit (StemCell Technologies) with minor modifications. At the organoid maintenance stage, half medium changes were automated by the Mo:re lab platform and bright-field images were collected to evaluate the organoid area and roundness. Moreover, at day 50, the cellular viability was evaluated using the CellTiter-Glo 3D Kit (Promega). As a control, the medium of another organoid plate was changed manually. We showed that the Mo:re lab platform performed the medium exchanges of cortical organoids without aspirating or disrupting the 3D structures. Further, the area and roundness of the cortical organoids were maintained during all medium exchanges demonstrating the accuracy and precision of the Mo:re pipetting technology. In addition, the organoids cultured with the automated device presented a higher cell viability than organoids cultured manually. In conclusion, the Mo:re lab platform enables the automation of medium exchanges of iPSCs-derived cortical organoids maintaining the 3D morphology. Therefore, this automated device represents a key tool for organoid standardization in high-throughput plate formats.

Title: Standardizing Long-term Cryopreservation on Patient-derived Organoids of the Gastrointestinal Tract

Author(s): Lara Zaidi, Viktoria Rösch, Mairene Coto-Llerena, Jens Grosch
Affiliation: Institute of Human Biology, F. Hoffmann-La Roche Ltd., Basel

Patient-derived organoids (PDOs) hold great potential as reliable and predictable 3D *in-vitro* models for pre-clinical research. The establishment of PDO lines from various tissue types and pathologies is well-established. However, further research is needed to develop effective methods for long-term cryopreservation.

It remains unclear how variables such as organoid size at cryopreservation, type of cryoprotective agents, and freezing methods, affect PDO recovery and viability. Here, we investigate qualitatively, by tracking organoid recovery after thawing, and quantitatively, with cell viability assays, how different cryopreservation techniques affect gastrointestinal PDOs. For standardization, we cryopreserved organoids at consistent sizes (40-60µm), and uniform density in *Recovery™ Cell Freezing Medium (ThermoFisher Scientific)*, supplemented with Y-27632 (10µM). A comparison between controlled-rate freezing and slow freezing in three PDO lines shows no significant differences in the recovery rate of organoids after short-term (<six months) cryopreservation at different time points. Consequently, we focused our long-term (<one year) cryopreservation experiments using the slow freezing method. We found that long-term storage of 16 gastrointestinal organoids did not significantly impact their recovery and viability. Overall, we observed comparable organoid growth and phenotypes after thawing two days versus one year after banking.

These findings suggest that standardization of cryopreservation conditions can enhance the robustness of gastrointestinal PDO models. Future research into PDOs from various tissue types and different culture conditions is essential for establishing standardized cultures.

Title

Blood-Generating Heart-Forming Organoids (BG-HFOs) recapitulate co-development of the human hematopoietic system and the embryonic heart

Authors

Miriana Dardano[1]✉, Felix Kleemiß[1], Maike Kosanke[2], Dorina Lang[1], Liam Wilson[1], Annika Franke[1], Jana Teske[1], Akshatha Shivaraj[3], Jeanne de la Roche[4], Martin Fischer[4], Lucas Lange[3], Axel Schambach[3, 5], Lika Drakhlis[1*]✉, Robert Zweigerdt[1*]✉

Affiliations

1 Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), REBIRTH–Research Center for Translational Regenerative Medicine, Hannover Medical School, Hannover, Germany. 2. Research Core Unit Genomics (RCUG), Hannover Medical School, Hannover, Germany. 3. Institute of Experimental Hematology, REBIRTH–Research Center for Translational Regenerative Medicine, Hannover Medical School, 30625 Hannover, Germany. 4. Institute for Neurophysiology, Hannover Medical School, Hannover, Germany. 5. Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA. ✉e-mail: dardano.miriana@mh-hannover.de; drakhlis.lika@mh-hannover.de; zweigerdt.robert@mh-hannover.de

*LD and *RZ contributed equally

Abstract

Deriving *in vitro* hematopoietic stem and progenitor cells (HSCs/ HPCs) is fundamental for therapeutic purposes yet challenging since their niche-like induction and stabilization in a proper developmental context is poorly understood. This is due to the limited knowledge of signals controlling these processes and the ethical concerns, since human embryogenesis is not easily investigable *in vivo*. Thus, a human *in vitro* approach properly resembling cellular and molecular aspects of hematopoietic development with neighboring tissues is of interest. Our recently established human pluripotent stem cell (hPSC)-derived heart-forming organoids (HFOs) represent a complex, well-patterned multi-tissue model recapitulating key aspects of the interconnected heart, vasculature and foregut development. Modulating HFO differentiation by the stage-specific supplementation of hemato-endothelial factors, we here report the generation of blood-generating-HFOs (BG-HFOs). While maintaining their heart anlagen, the organoids comprise specific, morphologically distinct endothelial subtypes including a mesenchyme-embedded endothelial layer giving rise to hematopoietic cells putatively via endothelial-to-hematopoietic transition (EHT). The *in vitro* model thus reflects central aspects of the intra-embryonic region that gives rise to hematopoiesis *in vivo*. Single-cell RNA sequencing of BG-HFOs revealed gene expression signatures resembling HPCs as well as hematopoietic derivatives including erythroid, megakaryocytic and myeloid cells. Functional assays revealed that BG-HFO-derived hemato-endothelial cells display erythroid, myeloid and lymphoid potential. Together, the data suggest that our model simultaneously presents aspects of both primitive and definitive hematopoiesis. The study reveals the first human model of self-organized, morphologically structured co-development of cardiac, endothelial and multipotent-hematopoietic tissues, providing a valid tool for pharmacological assessments, promoting research on hematopoiesis development *in vitro*, and overcoming limitations of such studies in the human embryo.

Apical-out airway organoids (AOAO):
a physiologically relevant model with high-throughput scalability for studying interactions between airborne viruses and the human respiratory epithelium

Mathieu Hubert[1], Paola Haemmerli[2,3], Loan Arthaud[1], Elodie Alessandri-Gradt[4], Georgios Stroulios[5], Yves Cambet[6], François Prodon[7], Bohumil Maco[1], Samuel Constant[8], Salvatore Simmini[5], Sophie Clément-Leboube[1], Antonio Grondin[9], Emerson Ferreira-Queiroz[1,2], Jean-Luc Wolfender[1,2], Caroline Tapparel[1]

[1] Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland.
[2] School of Pharmaceutical Sciences, Faculty of Medicine, University of Geneva, Geneva, Switzerland.
[3] Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland.
[4] CHU de Rouen, Université de Rouen Normandie UNI Rouen, Rouen, France
[5] STEMCELL Technologies UK Ltd., Cambridge, UK.
[6] READS Unit, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[7] Bioimaging Core Facility, Faculty of Medicine, University of Geneva, Geneva, Switzerland.
[8] Epithelix Sarl, Geneva, Switzerland
[9] Green Mission Department, Herbal Products Laboratory, Pierre Fabre Research Institute, Toulouse, France
Corresponding author: mathieu.hubert@unige.ch

The respiratory tract represents an entry site for numerous airborne viruses. In the airways, the epithelium is mainly composed of multiciliated cells, goblet cells, and pluripotent basal cells. In the laboratory, we implemented the differentiation of pluripotent basal cells in 3-dimensional respiratory organoids without the use of any extracellular matrix, resulting in the polarization of the ciliogenesis towards the exterior and the generation of apical-out airway organoids (AOAO). Due to the external exposure of the apical pole, these models are permissive to infection with different respiratory viruses in a way that mimics the apical exposure of the epithelium. We also assessed the development of AOAO from basal cells that were collected from diseased donors (asthma, cystic fibrosis, chronic obstructive pulmonary disease) and observed variations in cell composition and RSV replication in pathological AOAO, highlighting the role of the diseased epithelium in exacerbating respiratory infections. Moreover, we benefited from the scalability of AOAO to adapt experiments in a 384-well format to perform the first high-throughput screen of 800 plant extracts for their antiviral activity against RSV in a relevant model, after obtention of complementary data in A549 cells. From this screening, we identified approximately 60 extracts that effectively inhibited RSV infection. These findings will guide future research to isolate and comprehensively characterize the components that are responsible for antiviral activity.
Finally, apical-out airway organoids constitute a scalable and physiologically relevant model to investigate virus-host interactions and discover new potential therapeutic options to fight respiratory viruses of public health concern.

Keywords: airborne viruses, airway organoids, comorbidities, antiviral drug screen

A human iPSC-derived brain metastasis model to validate efficacy and toxicity of drug candidates

Krieg K[1], Materna-Reichelt S[2], Naber T[3,4], Woo MS[5], Siegmund M[6], Kauven P[1], Rachad FZ[1], Weller A[1], Haferkamp U[1], Wittich A[1], Zaliani A[1], Krasemann S[7,8], Stojanović Gužvić N[2], Wegener J[3,4] and Pless O[1]*

[1]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Discovery Research ScreeningPort, 22525 Hamburg, Germany
[2]Fraunhofer Institute for Toxicology and Experimental Medicine ITEM-R, 93053 Regensburg, Germany
[3]Fraunhofer Institute for Electronic Microsystems and Solid State Technologies EMFT, Cell-based Sensor Technology, 93053 Regensburg, Germany
[4]Institute for Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93053 Regensburg, Germany
[5]Institute of Neuroimmunology and Multiple Sclerosis, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany
[6]Center for Hybrid Nanostructures, University of Hamburg, 22761 Hamburg, Germany
[7]Institute for Neuropathology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany
[8]Core Facility for (Mouse) Pathology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany

**Presenting author*

Cancer metastasis results from the spread of disseminated cancer cells (DCCs) from primary tumors to distant organs and constitutes the primary cause of cancer-related mortality. Approximately 25% of cancer patients are affected by brain metastasis with severe neurological symptoms. However, current systemic therapies have limited efficacy and fail to improve survival rates. Consequently, there is an unmet clinical need to develop scalable human preclinical *in vitro* models that predict the interplay between metastatic cancer cells and the brain microenvironment to specifically explore therapeutic vulnerabilities of brain metastases. We have therefore established a production workflow for human induced pluripotent stem cell (iPSC)-derived cortical organoids in a screening-compatible 96-well format. To mimic brain metastasis, cortical organoids were exposed to melanoma cancer cells. Metastatic colonization of a fluorophore-labeled melanoma cell line was established and visualized by advanced imaging techniques. This workflow was extended to DCCs derived from lymph nodes of melanoma patients. Further, a comprehensive characterization of metastasis signatures was thoroughly conducted at mRNA and protein levels. To design a robust screening assay, we challenged these metastasis models with rationally selected and blood-brain barrier permeable tool compounds, achieving an assay window for efficacy and toxicity assessment. Finally, we utilized our melanoma brain metastasis platform to confirm and prioritize hit compounds identified in a drug screen using DCCs. In summary, our brain metastasis platform can serve as a complementary tool to validate and profile drug candidates with specific on-target efficacy against metastatic cancer cells and minimal off-target cytotoxicity on the niche forming brain tissue.

ADVANCING DISEASE MODELING TO DECIPHER THE CARDIORENAL PHENOTYPE OF FABRY DISEASE USING ORGANOIDS FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

Sandra D. Laufer[1,2], Anna Reinelt[1,2],Milagros N. Wong[1,2,3,4], Fabian Haas[1,2], Michaela Schweizer[5] , Linda Blomberg[6], Christine E. Kurschat[6], Nicola Wanner[1,2], Nicole Muschol[7], Victor G. Puelles[1,2,3,4], Arne Hansen[8,9], Tobias B. Huber[1,2], Fabian Braun[1,2,10]

[1]III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg
[2]Hamburg Center for Kidney Health (HCKH), University Medical Center Hamburg-Eppendorf, Hamburg
[3]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[4]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark
[5]Core Facility Morphology and Electron Microscopy, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf, Hamburg
[6]Department II of Internal Medicine, Center for Molecular Medicine Cologne and Center for Rare Diseases, University of Cologne, Cologne
[7]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg
[8]University Medical Center Hamburg-Eppendorf, Department of Experimental Pharmacology and Toxicology, Hamburg
[9]German Center for Cardiovascular Research (DZHK), Partner site Hamburg/Lübeck/Kiel
[10]Martin Zeitz Center for Rare Diseases, University Medical Center Hamburg-Eppendorf, Hamburg

Fabry disease is a multisystemic lysosomal storage disorder caused by mutations in the GLA gene leading to deficient or absent activity of the enzyme alpha-galactosidase A. Subsequent sphingolipid accumulation progressively affects the kidney, heart and nervous system. Our project aims to develop advanced human *in vitro* systems to improve our molecular understanding of the cardiorenal phenotype of Fabry disease which currently cannot be modelled accurately in rodents.
For this, we collected primary urinary cells from Fabry patients with various pathogenic variants. Following reprogramming into induced pluripotent stem cells (hiPSCs), we used CRISPR/Cas9 gene editing to create isogenic controls. Using published protocols, we differentiated these cell lines into heart and kidney organoids as well as cardiomyocytes for the generation of engineered heart tissues (EHTs).
We confirmed the loss of alpha-galactosidase A protein, deficiency of enzyme activity and the accumulation of globotriaosylceramide in patient-derived hiPSCs, organoids and EHTs. Treatment with enzyme replacement therapy and, when applicable, chaperone therapy resulted in reduction of substrate accumulation in the hiPSCs and both organoid systems. Fabry EHTs were prone to arrhythmias, reflecting the clinical phenotype of patients. Here, administration of enzyme replacement therapy again reduced substrate accumulation but did not alter the functional impairment. Furthermore, we confirmed the published pathological accumulation of synuclein alpha in Fabry nephropathy in kidney organoids.
In conclusion, we have established novel unique discovery platforms for Fabry disease. Ongoing experiments focus on single-cell analyses of the established systems in combination with deep proteomic and spatial phenotyping. Additionally, experiments investigate the involvement of the innate immune response, specifically the local production and activation of the complement system.

A human cerebral organoid model to investigate the host-pathogen determinants of Ebola virus persistence in the central nervous system

Widerspick, L.[1,2], Vidal Freire, S.[3], Steffen, J. F.[1], Shirsate, S.[3], Allartz, P.[1], Vickers, M. A.[1,2], Henkel, C.[1,2], Neira Pelen, P.[1,2], Nave, J.[1,2], Heung, M.[1,2], Wurr, S.[1], Müller, J.[1], Tappe, D.[1], Oestereich, L.[1,2], Crozier, I.[5], Worwa, G.[6], Kuhn, J. H.[6], Palacios, G.[3, 4] and Muñoz-Fontela, C.[1,2]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]German Center for Infection Research (DZIF), Partner site Hamburg-Borstel-Lübeck-Riems
[3]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[4]Global Health Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[5]Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, USA
[6]Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, MD, USA

Endemic in equatorial Africa, Ebola virus (EBOV), a member of the *Filoviridae* family, causes Ebola virus disease (EVD), a severe, systemic illness with an average case-fatality ratio of 45%. The recent characterization of long-term EBOV persistence in immune-privileged sites in EVD survivors has implications both for public health (i.e., reigniting and prolonging outbreaks) and for individuals (i.e., recrudescent inflammatory syndromes in survivors). EBOV persistence in the central nervous system (CNS) associated with severe meningoencephalitis has been rarely documented in human and nonhuman primate survivors; however, the host-virus determinants of CNS persistence are unknown. The current absence of tractable animal and *in vitro* models to investigate EBOV persistence critically limits experimental investigation. To address this gap, we present a partially immunocompetent human cerebral organoid model to explore EBOV persistence in a broad range of CNS host cells, including glial and neuronal populations. In this model, we investigated putative mechanisms for establishing and maintaining EBOV persistence at tissue and cellular levels, revealing microglia accumulation in sites of infection and astrocytes as late targets of EBOV. In cerebral organoids, EBOV persistently replicated for at least 120 days, accompanied with the release of pro-inflammatory markers such as CCL-2 and IL-6. Over time, we observed the accumulation of defective viral genomes along with naturally occurring EBOV variants. In long-term 2D *in vitro* culture maintained for 20 to 91 days, we further gathered evidence for a differential antiviral response in persistently infected microglia and astrocytes and cell-to-cell EBOV transmission among microglia and astrocytes, suggesting a multitude of host and viral mechanisms collaboratively determining EBOV persistence in the CNS.